6th Annual

Life Sciences Symposium

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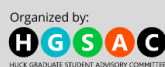
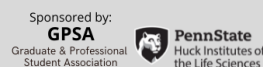Organized by:

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ABSTRACTS
Computational Biology & Statistics

#2 **The Reuse of Public Datasets: Potential Risks and Rewards from a Student's Perspective**

Alenka Hafner, Katharina Sielemann*, Boas Pucker*
Biology Department, Penn State University
*Genetics and Genomics of Plants, Center for Biotechnology, Bielefeld University

The 'big data' revolution has enabled novel types of analyses in the life sciences, facilitated by public sharing and reuse of datasets. Here, we review the prodigious potential of reusing publicly available datasets and the associated challenges, limitations and risks. Possible solutions to issues and research integrity considerations are also discussed. Due to the prominence, abundance and wide distribution of sequencing data, we focus on the reuse of publicly available sequence datasets. We define 'successful reuse' as the use of previously published data to enable novel scientific findings. By using selected examples of successful reuse from different disciplines, we illustrate the enormous potential of the practice, while acknowledging the respective limitations and risks. A checklist to determine the reuse value and potential of a particular dataset is also provided. The open discussion of data reuse and the establishment of this practice as a norm has the potential to benefit all stakeholders in the life sciences.

As a graduate student, I have encountered additional positive and negative aspects of reuse. I have access to vast datasets that were produced at immense cost and effort, however, these often do not allow for statistically robust re-analysis and/or have missing metadata. Ultimately, the rewards of reuse outweigh the risks and limitations. To ensure valid future reuse we must consider emerging data types and analysis tools to prevent more data from becoming unusable. We also need statistically meaningful experimental standards and community-implemented data standards.

#12 **The Secret Life of Molecules: Developing Extraction and Enrichment Systems For Cryo-EM Analysis of Natively-Sourced Assemblies**

Maria J. Solares, GM Jonaid, Deb F. Kelly
Biomedical Engineering, Penn State University

Tumor suppressor p53 is one of the most deregulated proteins in all oncological cases. In glioblastoma multiforme (GBM), the most common and deadliest forms of brain cancer, it is associated with poor prognosis events like tumor progression, invasion, and immortality. Unfortunately, little structural information is available on the N-terminal (NTD) and C-terminal (CTD) domains of p53. This has led scientists to develop conflicting theories on its regulatory mechanisms. The only information available is on the DNA binding domain (DBD), a stable region at the core of the monomeric protein that binds to DNA to seve as a transcription factor.

The lack of structural data on the "guardian of the genome" reflects a major trend in structural

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biology. Although X-ray crystallography has enabled scientists to discover major trends on macromolecules, the number of medical breakthroughs is limited by the capacity of current techniques. In the search of perfect crystals, unfortunately, scientists must cut out protein regions that are too flexible and disorganized to crystallize. On the other hand, cryo-electron microscopy (EM) may overcome these limitations. By relying on vitreous ice encapsulation, whole protein assemblies can be easily studied. However, to obtain a high-resolution structure, high concentrations of pure protein must be used. This has forced scientists to use recombinant protein expression systems. Yet, personalized medicine cannot rely on bacterial or yeast models devoid of disease-related modifications. We must explore assemblies from native sources to obtain real answers.

To overcome these limitations, we recently developed a highly reproducible extraction method coupled with a EM-specific enrichment step to image p53 assemblies from GBM cells. Our methods exploit the inherent nature of proteins and its post-translational modifications (PTMs) to bind metal cations like nickel-nitrilotriacetic acid (Ni-NTA), often used immobilized metal affinity chromatography (IMAC). Nuclear fractions of U87MG GBM cells were incubated with Ni-NTA agarose beads. These fractions were further concentrated for cryo-EM studies using lipid-base Ni-NTA-coated Silicon Nitride (SiN)-based microchips. Frozen p53 samples were inserted into a Talos F200C TEM operating at 200 kV. Images were collected using a CETA CMOS camera at low dose conditions (<5 electrons/A2/pixel) at ~92,000x magnification.

Single particle imaging processing using the RELION software package produced monomer reconstructions. The p53 monomer (~5 Å) initially accommodated a well-known DBD model but displayed never seen before extra density. A theoretical full-length p53 model was produced using PHYRE 2 protein prediction server and molecular modeling was refined using PHENIX and ISOLDE programs. By using a combination of rigid-body refinement and molecular dynamics-flexible fitting routines, we were able to validate the identity of the resolved p53 complex: a full-length, higher resolution p53 monomer. Through this model, we were able to demonstrate that our extraction and enrichment methods can produce enough particles for EM single particle analysis through. More importantly, our current work has been adapted to resolve patient-derived SARS-CoV-2 nucleocapsid protein, which has the same affinity for metal cations. These methods may be used to detangle the structure-function of several disease-related proteins and finally push the revolution needed for personalized medicine.

#21 **Can the enemy of my enemy be my friend? Lessons from field infections of two gastrointestinal helminths of the European rabbits**
Chiara Vanalli, Lorenzo Mari, Renato Casagrandi, Marino Gatto, Brian Boag, Isabella M. Cattadori
Biology Department, Penn State University

Natural animal populations are often infected with multiple parasite species that cause chronic infections and host morbidity. Within a host the interactions between coinfecting parasites are complex and can be either direct or indirect, for example mediated by the host immune response. These interactions can impact the dynamics of one or both parasite species, or can have no effect.

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Many of the interactions between single parasite species and the host immune response are well characterized, however, if and how these immune interactions might change and impact the infection dynamics when more than one parasite species are simultaneously infecting the same host, has not been adequately investigated.

Here, we used two common gastrointestinal helminths (Trichostrongylus retortaeformis and Graphidium strigosum) of European rabbits (Oryctolagus cuniculus) as a study system and asked: Does a second helminth species affect the intensity of the first helminth? What are the within-host immunological mechanisms that could generate the observed patterns of single and dual infections? To answer these questions, we used observations from a natural rabbit population, sampled monthly for six years (1/2005-12/2010). Data collected included parasite intensities of rabbits infected with one or both helminths and species-specific antibody IgA and IgG levels in the blood. We developed a within-host immune-infection model that describes rabbits with one or both helminths, and explicitly links parasite intensity and immune response by host age. We tested alternative mechanisms of parasite regulation in single infections and multiple immune-mediated interactions in dual infection, via a model selection approach.

Model selection showed that either IgA or IgG can explain the two helminth dynamics in single infections, although the antibody response is stronger against T. retortaeformis than G. strigosum. These findings are in agreement with previous laboratory infections, where similar immunological pathways against the two helminth species were observed in single infections. For dual infection, in addition to the reactions identified in single infections, we found evidence of a new cross-immune pathway, where the specific antibodies produced against the first helminth species are active in the regulation of the second helminth. This cross-immunological mechanism impacts the dynamics of the two helminth species in a disproportional way, as G. strigosum is more affected than T. retortaeformis. This asymmetric immune-mediated interaction was confirmed by laboratory infections, where rabbits were inoculated with both helminths. Additionally, we showed that the specific-specific antibody regulation against each helminth decreases when the other parasite is present, resulting in higher parasite intensities in dual infection compared to single infections.

In conclusion, when T. retortaeformis and G. strigosum are coinfecting the same host, the net effect of species-specific and cross- immunity against each helminth is lower than in single infections, indicating the immune-mediated interaction between the two helminths is beneficial for both parasites.

Our immune framework advances the mechanistic understanding of the mediated role of host immunity in multi-helminth infections and is flexible enough to capture a variety of immunological variables and their complex interactions.

#24 **Annual and non-annual cycles in the respiratory disease dynamics in tropical regions**
Fuhan Yang, Joseph L Servadio, Maciej F Boni
Center for Infectious Disease Dynamics, Department of Biology, Pennsylvania State University

Background: Respiratory diseases have been a research focus because they are one of the leading causes of global morbidity and mortality. Sufficient evidence has showed regular annual seasonality

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of influenza and other respiratory diseases in temperate regions. However, the seasonality of respiratory diseases in the tropics is less well-defined because of the lack of regular winter-forced environmental and behavioral change.

Methods: We built a community-based surveillance system including 89 outpatient clinics in Ho Chi Minh City, Vietnam, from 2010 to 2019. We monitored the patients that have the symptoms of respiratory diseases, defined as influenza-like-illness (ILI) using the daily messages sent from the clinicians. We detected the periodic signals of ILI time series using periodogram and wavelet spectra. We estimated the cycles using a simple cyclic step function. And we confirmed the existence of the cycles using regression models.

Results: We found there are both annual and non-annual (around 200 days) periodic signals present in the ILI time series. ILI activity showed 8.9% [95% CI: 8.8% - 9%] difference in the annual cycle, and 6.9% [95% CI: 6.6% - 7%] difference in the non-annual cycle, leading to all-year transmission pattern with 8.9% lower ILI activity from the beginning of March to the middle of May every year. The regression model predicts the ILI dynamics better when adding either of the cycle in the model, confirming the existence of both cycles. We also found that the 7-day autoregressive term of ILI, the annual cycle, and the non-annual cycle are the most important predictors predicting the ILI dynamics compared to climate factors and school term.

Conclusions: We found that the respiratory disease showed both annual and non-annual cyclic and weak fluctuations in a winter-absent tropical setting. The climate factors and the school term contribute less to the prediction of ILI dynamics, suggesting the lack of climate-forcing ILI dynamics in tropical regions.

#43 **Runs of homozygosity reveal extensive inbreeding among K'gari Island dingoes**

Ana V. Leon-Apodaca, Manoharan Kumar, Gabriel Conroy, Steven Ogbourne, Kylie M. Cairns, Sankar Subramanian, Zachary A. Szpiech
Biology Department, Penn State University

Dingoes (Canis dingo) are wild canids from an ancient canid lineage, now naturalized in Australia. Their evolutionary history remains contested, and it is thought that they arrived in Australia via South-East Asia at least 5,000 years BP, via one or more founder events. As Australia's largest native terrestrial predator, they play an important ecological role. They are found across many different bioregions of Australia, including on multiple offshore islands. A protected population exists on K'gari (Fraser Island) that is relatively free from the risk of hybridisation with domestic or wild dogs. K'gari is the world's largest sand island and a World Heritage listed national park. Over 600,000 people visit K'gari each year resulting in occasional negative human-dingo interactions. While many management strategies are in place to minimize this occurrence, lethal control has been utilized significantly in the past, and still   occasionally occurs. Previous research on K'gari dingoes using microsatellites, genome-wide SNPs, and mtDNA sequencing demonstrated divergence from mainland dingoes and low genetic diversity. However, whole-genome data is lacking from this important population. In this study, we analyze 18 whole genome sequences of dingoes sampled from mainland Australia (n=12) and K'gari Island (n=6) to assess the influence of their demographic histories on patterns of genetic diversity. Preliminary results showed that mainland dingoes and

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K'gari Island dingoes have distinct patterns of genetic diversity. We identify runs of homozygosity (ROH), indicators of small population size and inbreeding, in each population finding elevated levels of long ROH (>1 Mb) in both. However, K'gari dingoes showed significantly higher levels of very long ROH (>5 Mb; mean nROH = 71.2 for K'gari and 27.0 for the mainland, p = 5.35E-6; and mean sROH = 607.2 Mb for K'gari and 279.2 Mb for the mainland, p = 4.39E-4), providing clear evidence for inbreeding, isolation, small population size, and a strong founder effect. In the case of the K'gari dingo population, it appears that bottlenecks and isolation have maintained low levels of genetic diversity, while mainland dingoes show slightly higher diversity. We hypothesize that these ROH patterns may affect the distribution of deleterious homozygotes between mainland and K'gari dingoes. This work helps to elucidate the genetic structure and evolution of K'gari dingoes to inform conservation efforts.

Human Health, Nutrition, & Physiology

#8 **Predictive link between systemic metabolism and immune signaling in the brain of APOE4 mice**

Rebecca Fleeman, Amanda Snyder, Madison Kuhn, Dennis Chan, Amy Arnold, Elizabeth Proctor
Neurosurgery Department, Penn State College of Medicine

Alzheimer's disease (AD) is a neurodegenerative disease that results in memory impairment and cognitive decline. AD currently affects over 6 million Americans, where 1 in 9 people over the age of 65 will develop AD. While we do not fully understand why some people develop AD and others do not, we know the top risk factors for promoting AD pathology. The ε4 variant of apolipoprotein E (APOE) is the strongest and most common genetic risk factor for AD and is expressed by 25% of the population. While the mechanism of APOE4 conveyed risk is incompletely understood, promotion of inflammation, dysregulated metabolism, and protein aggregation have been implicated as potential contributions. AD is typically considered as a central nervous system disorder; however, AD patients also present with peripheral metabolic detriments. Previous studies have identified a link between systemic metabolism and brain electrophysiology changes, where communication between the hippocampus and periphery can involve autonomic innervation from the hippocampus to the pancreas and liver, as well as hippocampal signaling to the hypothalamus, a major metabolic control center in the brain. Here we asked how under-explored peripheral metabolic changes in APOE4 carriers may alter brain cytokine signaling to create a disease-promoting environment over the course of aging. We hypothesize that these detrimental effects exist even in the absence of AD-related proteinopathies, which develop in the brain only later in life. We identified the effects of systemic metabolic changes and brain immune signaling in young (3 mo) and aged (18 mo) female mice carrying the human APOE4 or APOE3 gene. Using a cross-disciplinary systems biology approach to combine results from functional metabolic assays and multiplex -omics profiling of hippocampal cytokine levels, we found that immune signaling patterns in the brain are predictive of systemic metabolic function. Specifically, the relationship between hippocampal cytokine signaling

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networks and systemic glucose tolerance differed by genotype: APOE4 mice produce lower levels of neuroprotective cytokines as their glucose tolerance declines. Similarly, APOE4 mice exhibited decreased neuroprotective cytokine secretion with increasing body fat percentage. To our knowledge, ours is the first study to identify a quantitative and predictive link between systemic metabolism and specific immune signaling signatures in the brain. Our results highlight the potential bidirectional relationship between systemic metabolic function and neuroimmune signaling, with this relationship becoming pathologically altered in APOE4 carriers to suppress neuroprotective pathways over the course of aging. Importantly, our findings indicate that consideration of the relationship between systemic metabolism and the neuroimmune state of the brain is crucial for a thorough characterization of the mechanism of APOE4 increased AD risk.

#30 **The Ancient Oral Microbiome in the Caucasus**
Sterling Wright[1], Marine Chkadua[2], and Laura Weyrich[1]
[1]Anthropology Department, Penn State University, [2]Georgia National Museum

Dental calculus, which is calcified dental plaque, from archaeological skeletons contains the DNA of ancient oral microbiomes. The oral microbiome refers to the collective DNA of microorganisms inhabiting the human mouth. To date, 700 species have been identified in the oral microbiome. Interest in studying the oral microbiome of archaeological populations has continued to grow because such examinations have provided novel insights into the population dynamics and life history of past peoples and their cultures. For instance, the analysis of ancient oral microbiomes in Europe has revealed that the number of disease-associated bacteria significantly increased during the Neolithic and Industrial Revolutions. However, whether similar shifts occurred in other geographic, temporal, and cultural contexts remain to be tested. This project addresses this research gap as it examines the ancient oral microbiome of ancient Georgia, a region in the Caucasus that remains poorly understood and largely uncharacterized. DNA sequencing technologies were applied to a total of 45 dental calculus samples dating to the Bronze Age (n=9), Antique period (n=9), and Middle Ages (n=25). The quality of the DNA sequences were checked using a custom bioinformatic script. Statistical analyses were performed in R to evaluate whether the microbial communities of each period were significantly different. The results indicate that indeed the communities were significantly different. Further analyses also identified oral species associated with each period. Interestingly, these analyses concluded that oral species that have been identified as key players in the development of periodontal disease (i.e., gum disease) were more abundant during the Antique period than the Bronze Age and Middle Ages. In conclusion, the current study reveals that the type of shifts that occurred in one geographic region did not universally occur. This means that studies aiming to understand how cultural transformations impacted human biology should take a micro-region approach as any broader scopes will overlook nuance differences. Furthermore, the results of this study suggest that such analyses can offer an additional framework in answering questions related to understanding the health of past populations.

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#37 **Perinatal High Fat Diet Alters Oxytocin Inputs onto Vagal Neurocircuits Controlling Gastric Motility**

Kaitlin E Carson, Jared Alvarez, Kirsteen N Browning
Neural and Behavioral Sciences, Penn State College of Medicine

Maternal overnutrition is known to impact the development of offspring, such as inducing metabolic and neurologic dysfunction, including increasing stress sensitivity. Perinatal high fat diet (pHFD) exposure has been shown to affect the development of vagal neurocircuits. Vagal neurocircuits, including the dorsal motor nucleus of the vagus (DMV) neurons, control gastrointestinal (GI) motility and exhibit plasticity to changes in diet and stress, leading to changes in gastric motility, tone, and emptying rates. Descending oxytocin (OXT; prototypical, anti-stress peptide) inputs from the paraventricular nucleus (PVN) of the hypothalamus synapse onto DMV neurons to modulate the GI stress response. However, how these descending OXT inputs, and associated changes to GI motility and stress responses, are altered following pHFD exposure are unknown. The present study was designed to investigate the hypothesis that pHFD reduces OXT inputs from the PVN to the DMV, altering DMV neuron responses and subsequent GI output to stress.

Rats were fed a control or high fat diet (14 or 60% kcal from fat, respectively) from embryonic day 13, and weaned onto the same diet at postnatal day 22. Male and female adult rats (N=7-8 control and pHFD) received microinjection of cholera toxin B, a retrograde neuronal tracer, into the DMV in order to quantify DMV-projecting PVN-OXT neurons. Another set of 8 rats (N=8 control and pHFD) were utilized to assess basal gastric emptying rates using the 13C octanoic acid breath test. Lastly, a subset of rats from both diet groups (N=4) underwent a 2-hour restraint stress, following which thin brainstem slices were collected to perform whole cell patch clamp electrophysiology on DMV neurons (N=6 cells/group) to assess the effect of OXT on GABAergic transmission. There was a significant reduction in PVN-OXT neurons projecting to the DMV in pHFD rats compared to controls (2.250 cells/mid-PVN coronal slice vs. 11.17 cells, respectively; $p < 0.05$ using unpaired t-test). Gastric emptying rates in pHFD rats were also significantly delayed compared to those fed a control diet (Half emptying time= 83.7 minutes vs. 60.1 minutes; $p < 0.05$ using unpaired t-test). Lastly, OXT had no effect on inhibitory GABAergic currents in control rats (101.547% baseline current frequency), but decreased inhibitory current frequency in stressed rats OXT (54.17% baseline current frequency). In contrast, OXT increased inhibitory currents in pHFD rats (182.592% baseline current frequency), an effect that was lost following stress (105.85% baseline current frequency).

These results indicate that pHFD exposure reduced OXT innervation to the DMV and these changes may lead to delayed basal gastric emptying rates. Together with alterations in the response of DMV neurons to OXT, these results suggest pHFD may lead to a maladaptive GI stress response and contribute to reduced stress resiliency in offspring.

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Microbiology, Pharmacology, & Immunology

#1 Metformin modulates the microbiome of broiler breeder hens in an avian model of ovarian dysfunction.

Emily Van Syoc, Evelyn Weaver, Connie Rogers, Justin Silverman, Ramesh Ramachandran, Erika Ganda
Integrative & Biomedical Physiology and Clinical & Translational Sciences Dual-Title PhD Program, Penn State University

Broiler breeder hens are the parent stock of commercial broiler chickens, which are genetically selected for rapid growth. Since broiler breeders outlive the production lifespan of broiler chickens, these traits result in excess adiposity and severe ovarian dysfunction in a phenotype that mimics human polycystic ovarian syndrome (PCOS). Metformin is a drug indicated for type 2 diabetes but is widely prescribed off-label for PCOS. Clinical trials of metformin for human PCOS have resulted in decreased obesity and androgens, and improved pregnancy rates. Metformin improves insulin sensitivity by decreasing hepatic gluconeogenesis and thereby blood glucose levels, but metformin also modulates the gut microbiome, which lowers circulating lipopolysaccharide and reduces toll-like-receptor-mediated inflammation. The effects of metformin on broiler breeder hens have not been tested, thus we hypothesized that metformin improves ovarian dysfunction partially through modulation of the gut microbiome. A trial of four metformin doses (0, 25, 50, or 75 mg/kg body weight) from 25 through 65 weeks of age was performed to determine if metformin improves the metabolic and reproductive health of broiler breeder hens. A subset of hens (n=8-10) was randomly selected from a larger cohort to undergo longitudinal microbiome profiling with 16S rRNA sequencing. Metformin prolonged the production lifespan; 70% of the hens on 75 mg/kg metformin were still laying at 65 weeks compared to 44% of hens in the control group ($p < 0.05$). Microbial community structure (beta diversity) differed between 50 mg/kg and 75 mg/kg ($p = 0.002$) and between 25 mg/kg and 75 mg/kg ($p = 0.001$) but not between 25 mg/kg and 50 mg/kg ($p = 0.42$). In addition, metformin-treated hens had significantly different community structures between 40 and 60 weeks of age ($p = 0.005$) and between 50 and 60 weeks of age ($p = 0.006$), but not between 40 and 50 weeks of age ($p = 0.338$). Differential relative abundance analysis revealed 34 bacterial genera with significantly higher and 8 genera with significantly lower relative abundance in 75 mg/kg metformin than the control treatment (0 mg/kg metformin). These results demonstrate that metformin has a significant impact on the microbiome of broiler breeder hens in addition to significant interactions with hen age. Metformin improved the production lifespan which correlated with perturbations to the gut microbiome, demonstrating a potential beneficial effect of metformin in broiler breeder hens.

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#3   Oxygen-dependent Signaling by Globin Coupled Sensors in Pathogenic Bacteria
Florian Fekete, Emily Weinert
Department of Biochemistry and Molecular Biology, Penn State University

Bacteria sense and respond to numerous environmental cues, such as oxygen due to its importance in respiration. A family of oxygen sensors, termed globin coupled sensor (GCS) proteins, are found in a wide range of bacteria and consist of a heme-containing globin domain that is connected through a linker domain to an output domain. One such output is a diguanylate cyclase domain, which synthesizes cyclic-di-GMP (c-di-GMP), an important bacterial second messenger, upon oxygen binding to the heme. Functions known to be regulated by c-di-GMP include motility, biofilm formation, and virulence factor secretion, but the cellular effects of oxygen sensing through diguanylate cyclase-containing GCS proteins have not been widely studied in vivo.

The role of diguanylate cyclase-containing GCS proteins is being studied in the soft rot plant pathogen Pectobacterium carotovorum. P. carotovorum is an ideal model, as plants are known to use oxygen in their wound response and low oxygen levels often correlate with virulence phenotypes in plant pathogens. Initial studies identified phenotypic effects of O2-sensing by the GCS protein, including secretion of exoenzymes, modulation of N-acylhomoserine lactone (AHL) levels, and altered motility in P. carotovorum. To further probe the signaling mechanism by which the GCS alters P. carotovorum phenotypes, this work employs a transcriptomics and phenotypic assays using a GCS deletion strain of P. carotovorum to provide further insight into functions controlled by GCS proteins. Additional studies are investigating the role of protein-protein interactions in GCS signaling within P. carovotorum. Finally, insights from P. carotovorum GCS signaling are being applied to investigate GCS signaling in E. coli, which has previously been shown to be associated with the RNA degradosome. Taken together, these studies improve our understanding of the roles of c-di-GMP-producing GCS proteins and further understanding of signaling within and important plant pathogen and other GCS-containing Gram-negative bacteria.

#14   A synthetic microbiota designed via meta-analysis exhibits C. difficile colonization resistance
Susan Tian, Jordan Bisanz
BMMB Department, Penn State University

Clostridioides difficile (Cd) is a common cause of recurrent antibiotic-associated diarrhea and colitis for which long-term efficacious and safe treatments are needed. Cd is normally suppressed by a healthy gut microbiome but flourishes due to the off-target effects of antibiotics disrupting microbiome composition. Antibiotics are initially effective in treating CDI; however, their persistent disruption to the microbiome frequently leads to recurrent infection. Fecal microbiota transplant (FMT) has been shown to be an effective treatment but the reliance on human donors makes FMT composition intrinsically irreproducible. FMT may have undesirable adverse effects

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on host physiology and/or transmit undesirable microbes such as multidrug-resistant pathogens. As a solution, we designed a synthetic FMT (sFMT) using meta-analysis of 12 human studies wherein Cd colonization was clinically determined (N=899 samples). Microbiome composition could robustly predict Cd colonization status with an underlying set of taxa robustly anti-correlated with Cd. We built a lab-derived sFMT from 39 tractable strains and found it was capable of stably colonizing gnotobiotic mice offering resistance to colonizationn with Cd 630. A variant of the sFMT with limited ability to form 2° bile acids retained efficacy demonstrating that the sFMT functions independently of this well-known Cd-antagonistic trait. Taken together, our data demonstrate that meta-analysis is a viable strategy to design ecologically-informed synthetic microbiota which will allow for further mechanistic investigation into the mechanisms of the microbiome in health.

#19 **Comprehensive mapping of bile acid metabolism by the gut microbiome**
Min Soo Kim, Jordan E Bisanz
Biochemistry, Microbiology and Molecular Biology (BMMB), Penn State University

A growing body of evidence implicates the gut microbiome in host pathophysiology through the exchange of small molecules with each other and the host. A key family of metabolites are bile acids (BA). Primary BAs are the products of cholesterol metabolism in the liver and serve as emulsifiers that aids in uptake and clearance of lipids in the intestinal lumen. However, commensal microbes in the gut can convert primary bile acids to secondary bile acids through deconjugation, oxidization, epimerization, and dehydroxylation. Primary and secondary BAs, as well as their conjugated and unconjugated derivatives can activate the expression and function of key host nuclear receptors, such as FXR and PXR, and modulate inflammation through RORγt. These can impact downstream signaling that influences host health and diseases. Indeed, disruption of microbial bile acid metabolism is correlated with marked changes in the microbial composition and accompanying development of metabolic diseases, inflammatory bowel diseases (IBD), C. difficile infection (CDI), and colorectal cancer. Despite their importance in health, we have a limited understanding of which microbes perform these reactions in complex communities. In addition, of over hundred bile acids identified in host intestinal tissues and feces, only a small subset has known functions and origins. To understand the mechanistic basis for how microbes manipulate the host through bile acid metabolism, and to move from correlation to causation, we require a high-resolution map of which microbes and pathways are responsible for the varied reactions in the gut. I hypothesize that interpersonal variance in the gut microbiome composition shapes the bile acid pool, which in turn confers differential resistance to C. difficile infection in host.

To test my hypothesis, I plan to screen the library of 352 isolated bacterial strains by inoculating them in media supplement with a consortium of bile acids and analyzing metabolism via LC/MS. This will allow identification of microbial species that can metabolize bile acids and determination of which specific biochemical reaction those species undertake. Preliminary results

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show that distinct metabolic profiles arise when different isolated strains are grown in the presence of bile acids. A high throughput screening method to determine bile acid modulating ability of microbes is currently being developed. The sequences of strain identified to metabolize bile acids will be analyzed by comparative genomics to discover metabolic pathways for bile acid transformations. Gene functions will be validated using the knock-in approach. Lastly, I will investigate the effect of the composition of bile acid modulating microbes on the resistance to C. difficile in a mouse model of recurrent infection. I will gavage different consortiums of BA metabolizing microbes into germ-free mice and challenging them with C. difficile. I hypothesize that increasing the ratio of secondary bile acid producing microbes will increase CDI resistance.

#27 **Antimalarial Modulation of the Human Gut Microbiome**
Benjamin Anderson, Min Soo Kim, Jordan Bisanz
Biochemistry, Microbiology and Molecular Biology (BMMB), Penn State University

The World Health Organization reports that in 2020 there were 241 million cases of malaria and 627,000 malaria related deaths. Malaria is caused by parasitic Plasmodium species, and one common treatment is the use of antimalarial drugs that disrupt the Plasmodium life cycle; however Plasmodium can quickly develop resistance to these drugs. Inadequate blood serum levels of antimalarial drugs can contribute to the development of antimalarial resistance in Plasmodium, so proper dosing is essential. Antimalarial drugs show high levels of interpersonal variation and have poor bioavailability, suggesting a role for environmental factors including microbiome composition. As orally administered drugs, we hypothesize that antimalarials can both be modified by and alter the composition of the human gut microbiome altering drug absorption and thus can alter the treatment efficacy and/or increase the rate of development of drug-resistant Plasmodium species. The goal of this project is to determine how the gut microbiome interacts with antimalarial drugs, hydroxychloroquine and artemisinin. Through ex vivo experiments using human fecal donors we have demonstrated that antimalarial drugs have off-target antimicrobial effects on the gut microbiome with a high degree of interpersonal variation among donors. To identify microbes which are susceptible and resistant, we used 16S rRNA gene sequencing to determine the strains that have differential growth in the presence of drugs. Using drug-resistance as a proxy for possible inactivating metabolism, we are now using metabolomic methods to uncover potential metabolic products and determine which microbial pathways lead to their creation. Going forward, we plan to expand these studies into in vivo animal models to demonstrate biological significance of antimalarial-drug interactions and generate a comprehensive strain-level understanding of drug-microbe interactions in the gastrointestinal tract.

#28 **Evaluation of Oncoselectivity and Oncotoxicity of Novel Oncolytic Viruses in vitro**
Abirami Ravichandran, Ruth H. Nissly, Padmaja Jakka, Meera Surendran Nair, Shubhada K. Chothe, Marco Archetti, and Suresh V. Kuchipudi
Department of Integrative and Biomedical Physiology, Penn State University

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Significance and purpose

Oncolytic virotherapy is a mode of cancer therapy involving the usage of replication-competent viruses to kill cancer cells. Oncolytic viruses (OVs) effectively lyse cancer cells while causing minimal or no effect on normal cells. Many natural and recombinant OVs are currently being studied in clinical trials against a wide range of cancers. However, the presence of preexisting immunity against most of these viruses limits their efficacy. To address this limitation, we discovered two novel oncolytic viruses, namely, OV1 and OV2, which are animal viruses that are non-pathogenic to humans. Humans do not have preexisting immunity against these viruses. Preliminary studies suggested that OV1 and OV2 possess oncolytic activity. This study characterized the oncoselectivity and oncotoxicity of OV1 and OV2 in vitro.

Methodology

We characterized the oncoselective and oncotoxic properties using a range of human cancer and normal cells. Human renal (769P, Caki2), mammary (HCC1187, HCC1500), colorectal (HCT116, Caco2), liver (Huh7.5) cancer cells, and leukemia (THP-1) cells, immortalized embryonic kidney cells (293T), fetal lung fibroblasts (MRC-5), and primary bronchial epithelial cells were infected with OV1 and OV2 at multiplicity of infection (MOI) of 0.001, 0.01, 0.1, 1, 2.5, and 5. After 72h of OV infection, MTS assay to determine cell viability and Caspase-Glo 3/7 assay to quantify apoptosis were performed. Additionally, the cells were infected at MOI of 0.1, 1, and 5 to study the viral replication by qRT-PCR and observe cytopathic effects (CPE) and the presence of viral protein in infected cells by immunofluorescent staining.

Results

After infection with OV1, there was significantly decreased viability and increased apoptosis of cancer cells (HCC1187 at MOI$\geq$0.001, HCC1500 at MOI$\geq$0.1, 769P at MOI$\geq$0.1, and Caco2 at MOI$\geq$0.01; $p<0.05$). Post-infection with OV2, there was a significant decline in the viability and increase in apoptosis of cancer cells (HCC1500 at MOI$\geq$0.001, HCC1187 at MOI$\geq$0.1, 769P at MOI$\geq$0.001, Caki2 at MOI$\geq$0.1, HCT116 at MOI$\geq$0.1, Huh7.5 at MOI$\geq$1, and Caco2 at MOI$\geq$1; $p<0.05$). The viability and apoptosis levels of immortalized and normal cells were not significantly affected by OV treatment, except at very high doses. Further, we observed an increase in the viral replication of OV1 and OV2 in the tumor cells. Cell death and CPE were observed through microscopic examination in the cancer cells after OV infection. Additionally, the presence of intracellular viral protein was detected using immunofluorescent staining. However, in normal and immortalized cells, cytopathic effects, cell death, and the presence of viral protein were not observed.

Conclusion

The above findings provide solid evidence that OV1 and OV2 possess cancer-selective replicative (oncoselective) and lytic (oncotoxic) properties. The results also demonstrate that they do not affect normal cells. These observations suggest that OV1 and OV2 could be potential candidates for oncolytic virotherapy. Further, the in vitro studies suggest an enhanced susceptibility of breast cancer to OV treatment. Thus, a follow-up experiment to characterize the in vivo oncolytic effects of OVs will be performed in a murine breast cancer model. Additional

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studies are underway to generate recombinant viruses with enhanced oncolytic and immunostimulatory properties.

Molecular Biology, Genetics, & Chemistry

#5 **Ligand-specific mechanisms of allosteric regulation in FXR**
Tracy Yu, Noriko Mikeasky, Emily Meinert, Namita Dube, C. Denise Okafor
BMMB Department, Penn State University

Farnesoid X receptor (FXR) is a member of the nuclear receptor family of ligand-regulated transcription factors that regulates bile acid, lipid and glucose metabolism. Bile acids are the endogenous FXR ligands. Bile acid binding to FXR induces association with FXR response element sequences (FXREs), and promotes selective recruitment of coregulator proteins via induced structural changes in the activation function AF-2 surface, i.e. the site of coregulator binding. However, there is a poor understanding of how FXR is allosterically regulated by bile acid, which acts as a molecular switch to initiate critical long-range of transcriptional events. The goal of this work is to understand how minor structural modifications in bile acids give rise to differential FXR transcriptional activity. We used dual luciferase assays to determine how bile acids modulate FXR DNA binding preferences for a a wide range of FXREs. We also used molecular dynamics (MD) simulations to study the effect of the bile acids on AF-2 signaling. We have identified key residues that mediate specific allosteric communication pathways in FXR, and the mutations of the key residues in FXR-LBD affects bile acid-specific FXR transcriptional activities, confirming their roles in ligand-AF-2 allostery. These findings can further our understanding of how bile acids impact FXR promoter selectivity and coregulator recruitment**.**

#11 **Nontyphoidal Salmonella isolated from dogs reveal antimicrobial resistance determinants and relatedness to strains found in humans**
Sophia Kenney, Nkuchia Mikanatha, Erika Ganda
Animal Science Department, Penn State University

Background
Non-typhoidal Salmonella (NTS) is a cause of foodborne illness in the United States, leading to outbreaks, food recalls, and economic losses. Of the estimated 1.35 million yearly infections, 212,500 are linked to antimicrobial resistant (AMR) Salmonella strains. This increasing AMR combined with the wide host range of NTS creates overlapping challenges for human and animal health, and the need for biosurveillance and outbreak tracking. Typically, human infections are acquired through consumption of contaminated food or contact with animals. Although zoonotic origin is known, comparison of AMR determinants in NTS

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clinical isolates from dogs and humans is limited. An estimated 48 million and 7.5 million households in the United States and Canada, respectively, own one or more dogs. However, AMR determinants in NTS clinical isolates from dogs and genetic relatedness to strains found in humans is unclear.

Methods
Sixty-three NTS isolates from companion dog sources and 16 human clinical isolates were identified through NCBI's Pathogen Isolate Browser and collaboration with the Veterinary Laboratory Investigation and Response Network, a network of diagnostic laboratories spanning the United States and Canada responsible for investigating animal illness outbreaks and tracking AMR bacteria from sick animals. Core genome MLST, 7-gene-MLST, and SNP-based clustering schemes were used to compare strains collected from 2017-2021. AMR traits included in NCBI isolate metadata were compared within clusters.

Results
AMR determinants for antibiotic efflux capabilities were identified in 95% of isolates, with genes associated with resistance to tetracyclines, sulfonamides, and aminoglycosides also present in specific clusters such as one of S. Typhimirium isolates. Of the 16 human isolates included, 14 clustered with those from dogs regardless of clustering scheme.

Conclusions
Whole-genome sequencing has proven to be a useful epidemiological tool to investigate illness outbreaks and monitor AMR genetic elements in relevant pathogens. The strain-relatedness observed here supports the potential for NTS zoonosis between dogs and humans. Furthermore, the presence of AMR genes in isolates across sources substantiates the need for biosurveillance across a range of NTS reservoirs.

#15 **Enzyme Aggregation and Fragmentation**
Kayla Gentile, Ashlesha Bhide, Joshua Kauffman, Subhadip Ghosh, Subhabrata Maiti, James Adair, Tae-Hee Lee, Ayusman Sen
Chemistry Department, Penn State University

Protein aggregation has been implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Additionally, aggregation of proteins in vitro can affect certain experimental observations, especially those that rely on protein size. An example of this is the study of enhanced diffusion of proteins that catalyze reactions, also known as enzymes. Multiple studies have reported that certain enzymes show higher diffusion in the presence of their substrates. However, since size is related to diffusion according to the Stokes-Einstein equation, aggregation or fragmentation of enzymes could affect these conclusions. In this presentation, I will talk about our study where we show how certain enzymes aggregate or fragment in the presence of chemicals relevant to their catalysis. We investigate three

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enzymes, alkaline phosphatase, hexokinase and glucose oxidase and employ techniques such as fluorescence resonance energy transfer (FRET), dynamic light scattering (DLS) and atomic force microscopy (AFM) to study the aggregation and fragmentation of these enzymes. We found that alkaline phosphatase aggregates upon addition of zinc ions and inorganic phosphate. We also found that the two enzymes that react with glucose, hexokinase and glucose oxidase, fragment in the presence of D-glucose. These results suggest that these enzymes do not retain their native structure during catalysis. We are also interested in taking this one step further and using these enzyme properties to design biocompatible drug-delivery vehicles that can aggregate upon addition of certain chemicals. Thus, I will also talk about my current project which involves synthesizing vesicles that are coated with enzymes and studying whether they aggregate and fragment similar to the native enzyme molecules. These aggregation studies provide new insights into the structure of native proteins and also give us a way to control the motion of potential drug-delivery vehicles.

#18 **Mechanical regulation of histone H3 lysine 9 methylation during TGFβ1-induced Epithelial-Mesenchymal Transition**

Chinmay S. Sankhe, Matthew J. Bierowski, Jacob A. Karnick, Rachel L. Cecco, Esther W. Gomez
Department of Chemical Engineering, The Pennsylvania State University

Epithelial-mesenchymal transition (EMT) contributes to regulation of normal physiological processes including embryogenesis, organ development and wound healing, and is also associated with pathological conditions such as fibrosis and cancer. During EMT, epithelial cells lose cell-cell interactions and begin to exhibit a mesenchymal-like phenotype with increased cell motility and invasion into the surrounding extracellular matrix. Thus, it is important to study the molecular mechanisms governing the EMT process in order to develop strategies to target this pathway in pathological contexts. The stiffness of the extracellular matrix mediates EMT, with an increase in matrix stiffness promoting loss of epithelial characteristics such as cell-cell contacts, polarity and E-cadherin protein expression along with enhancing mesenchymal traits such as cell motility and remodeling of the cytoskeleton. EMT is accompanied by dramatic alterations in gene expression patterns in part due to modifications to the chromatin architecture including histone modifications. It has been shown in the literature that methylation of lysine 9 on histone H3 (H3K9) is associated with the silencing of the epithelial protein marker E-cadherin during EMT. However, there is limited knowledge on how the mechanical properties of the extracellular matrix, specifically matrix stiffness, modulates histone modifications during EMT. We hypothesize that matrix stiffness regulates H3K9 methylation during EMT. In order to mimic the cellular microenvironment, we fabricated hydrogels spanning the stiffness of normal and diseased mammary tissue and monitored the response of mammary epithelial cells to transforming growth factor (TGF)-β1 induced EMT. Using assays such as immunofluorescence staining and western blotting, we demonstrate that matrix stiffness and TGFβ1 treatment together modulate the bulk levels of

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H3K9 methylation markers. We also find that inhibiting the activity of histone lysine methyltransferases, protein enzymes that catalyze the addition of methyl groups to amino-acid residues on histone tails, modulates EMT-associated changes in response to matrix stiffness. Furthermore, treatment with cell contractility inhibitors modulates the bulk levels of H3K9 methylation in response to EMT induction cues and matrix stiffness. These findings suggest a regulatory role for extracellular matrix mechanics and cytoskeletal contractility in the modulation of EMT via control of histone modifications. These observations may further suggest strategies to prevent EMT in pathological contexts including fibrosis and cancer.

#23 **Kinetic asymmetry determines the direction of enzyme chemotaxis**

Niladri Sekhar Mandal, Ayusman Sen, R. Dean Astumian
Chemical Engineering Department, Penn State University

Enzyme chemotaxis is the directional motion of enzyme molecules towards or away from a gradient of their respective substrates and products. The phenomenon has wide ranging implications for not just the modern-day applications, such as drug delivery and disease detection, but also for determining the physiochemical driving force behind origin of life and its subsequent evolution. While there have been several hypotheses on why enzymes chemotax, the underlying principle remains to be fully elucidated. In this study, we propose a mechanism of chemotaxis based solely on the diffusion and chemical kinetics of the enzyme molecules. We show two factors. 1) kinetic asymmetry, the difference between the unbinding rates of the substrates and the products and 2) diffusion asymmetry, the difference in the diffusivities of the unbound and the bound form of the enzyme, govern the direction of enzyme chemotaxis. Our model captures the non-equilibrium distribution of enzyme molecules while they maintain complete mechanical equilibrium with the surroundings and thus fulfills the criterion of low Reynold's number regime.

We begin our analysis with a uniform concentration of an enzyme and a constant gradient of substrate and product in the domain. We use trajectory thermodynamics to determine the role of kinetic and diffusion asymmetry in determining the direction of enzyme chemotaxis. We confirm the role of kinetic asymmetry by solving rate equations for the enzyme and obtaining the steady state enzyme distribution. Furthermore, it has been suggested in literature that in evolution of simple matter into complex life-like structures, the most favored state is the one that has the maximum rate of energy dissipation. Our work is important in this context, as we have been able to show that the rate of dissipation plays no role in determining the direction of chemotaxis. This allows us to conclude that kinetic and diffusion asymmetry, and not rate of dissipation, is the controlling factor for how matter evolves from simple states to complex ones.

#31 **Systematic dissection of sequence features affecting binding specificity of a pioneer factor**

Cheng Xu, Holly Kleinschmidt, Jenna Johnson, Lu Bai
Biochemistry and Molecular Biology Department, Penn State University

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Sequence-specific transcription factors (TFs), which recognize their cognate DNA motifs, are central players in regulation of gene expression. In higher eukaryotes, transcription factors (TFs) only bind to a small proportion of their binding motifs across the genome, partially because of the inhibition of nucleosomes. A group of TFs named pioneer factors (PFs) are able to overcome the barrier and bind to nucleosomal DNA. However, the fraction of motifs occupied by PFs in the genome is still very small. Both chromatin background and local DNA sequence features can contribute to this site selectivity, yet the exact effect of individual features remains undefined. Moreover, Current methods, mostly based on studying binding sites across the genome to tackle this problem, can hardly distinguish between various factors and establish causal relationship. In order to systematically dissect local sequence features affecting PF binding in the same chromatin background, we designed a high-throughput assay named Chromatin Immunoprecipitation with Integrated Synthetic Oligonucleotides (ChIP-ISO). This method involves integrating thousands of different synthetic sequences containing PF motifs into a fixed genomic locus in mammalian cells, followed by chromatin immunoprecipitation (ChIP) and next-generation amplicon sequencing. We applied ChIP-ISO to studying the binding specificity of FoxA1, a classic PF essential for cell differentiation and cancer development, in A549 human lung carcinoma cells. By interrogating the enhancer of CCND1 gene, we found that within the same sequence background, FoxA1 binding is strongly affected by its motif strength, motif orientation, number of motifs and co-binding TFs including AP-1 and CEBPB. AP-1 is particularly important for enhancing FoxA1 binding to both CCND1 enhancer and genome-wide loci, which is further illustrated by mutating AP-1 motifs in FoxA1-binding sites across the genome through ChIP-ISO, inhibition of AP-1's DNA binding activity and in vitro DNA-protein binding assay. Our results also indicate that in the cellular content, relatively low expression of FoxA1 in combination with presence of certain co-binding TFs may lead to cell-type-specific binding patterns. Finally, by moving sequences originated from different genomic loci to the same chromatin background and measuring FoxA1 binding, we showed that FoxA1's binding specificity is more determined by the local sequence than chromatin background. In summary, our study provides insights of the genetic rules underlying PF binding specificity and reveals a potential mechanism to regulate its binding events during cell differentiation. Our study also establishes a paradigm for understanding TF binding specificity and cis-regulatory logic, applicable to the characterization of designed sequences and human genetic variations.

#32 **Schizophrenia risk gene ZNF804A regulates Parvalbumin and Perineuronal Net Expression**

Shray Patel, Zifei Pei, Yingwei Mao
Biology Department, Penn State University

Schizophrenia is a chronic neurological disorder showing specific cellular and brain structural changes. Most parvalbumin (PV) positive interneurons are surrounded by perineuronal nets

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(PNNs), a component of the extracellular matrix. PNNs associated with PV interneurons in the prefrontal cortex have been shown to have decreased immunofluorescence intensity in postmortem brains of schizophrenia patients. Previously, our lab has shown that a schizophrenia risk gene, zinc finger protein 804A (ZNF804A), regulates translational machinery in the developing brain. To understand how ZNF804a modulates PV and PNN expression, we used a CRISPR-cas9 to knock out the mouse orthologue of ZNF804A, Zfp804a. Confocal microscopy was used to quantify fluorescence intensities of PNNs–immunolabeled with Wisteria Floribunda Aggrecan (WFA)–and PV neurons. Immunostaining times and antibody concentrations were standardized across samples, and each staining batch contained multiple genotypes to avoid confounding. We found that Zfp804a knockout mice have reduced PV intensity (p = 0.04, Hedge's g = -2.11) and PNN intensity (p = 0.03, g = -2.22) in layers 4 and 5 of the prefrontal cortex. Surprisingly, we also demonstrate that Zfp804a knockout mice have lower PV (p = 0.02, Hedge's g = -1.41) and PNN intensity (p = 0.03, Hedge's g = -2.09) in the thalamic reticular nucleus. Because PV neurons have been identified to regulate the excitatory/inhibitory balance in the brain, the observed alterations in PV expression may explain the disruptions of excitatory/inhibitory balance identified in schizophrenia. Because PNNs modulate PV cellular development, decreases in both PV and PNN expression may also indicate that thalamo-cortical circuits are disrupted in subjects that lack Zfp804a, elucidating a possible mechanism of schizophrenia pathogenesis.

#35 **Autophagy enhances the intestinal epithelial tight junction barrier by upregulating cellular occludin levels and enhancing its localization to the paracellular Tight Junction.**
Kushal Saha, Ashwinkumar Subramenium Ganapathy, Alexandria Wang, Nathan Morris, Eric Suchanec, Gregory Yochum, Walter Koltun, Wei Ding, Meghali Nighot, Thomas Ma and Prashant Nighot.
Department of Medicine, Penn State College of Medicine.

Background and Aim: Loss of paracellular tight junction (TJ) barrier function of the gut epithelium is associated with inflammatory bowel disease (IBD). Defects in autophagy genes are risk factors potentiating IBD. Our previous studies showed the TJ barrier enhancing the role of autophagy, however, its role in the regulation of the barrier-forming protein occludin remains unknown. Here, we investigate the role of the autophagy pathway in the regulation of occludin and its role in inflammation-mediated barrier loss.
Methods: Pharmacological and genetic tools were used to study the effect of autophagy on occludin levels and localization, and the role of the MAPK pathway.
Results: Autophagy induction using pharmacological activators and nutrient starvation increased total occludin levels and reduced large molecule inulin flux in Caco-2 cells. Nutrient-starvation enriched membrane occludin levels in Caco-2 cells and mouse colonoids and also phosphorylation of Thr residues on the cytoplasmic tail of occludin. Starvation-induced TJ barrier enhancement was contingent on the presence of occludin as OCLN-/-nullified the TJ barrier enhancing effect of starvation. Autophagy inhibited the lysosomal

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targeting of occludin thus enhancing its half-life and protecting against inflammation-induced TJ barrier loss. Starvation-induced TJ barrier enhancement was prevented by inhibition of autophagy. Our study also highlights the role of the MAPK pathway on autophagy-mediated occludin upregulation. Autophagy enhanced the phosphorylation of ERK-1/2. Inhibition of these kinases in Caco-2 cells and surgically resected human intestinal tissue inhibited the protective effects of autophagy. In-vivo, autophagy induction increased occludin levels in mouse intestines and protected against LPS and TNF-α-induced TJ barrier loss. Additionally, acute Atg7 knockout in adult mice decreased intestinal occludin levels, increasing baseline colonic TJ permeability and exacerbating the effect of DSS-induced colitis.

Conclusion: Our data suggest a novel role of autophagy in promoting the intestinal TJ barrier by reducing degradation and increasing membrane localization of occludin, in a MAPK-dependent manner.

#36 **Interface-induced miRNAs from Cuscuta campestris are detectable early during infection, and may be transcribed by RNA POL III.**

Collin Hudzik, Mike Axtell
Biology Department, Penn State University

Cuscuta campestris is a holoparasitic stem parasite which lacks leaves, roots, and enough chlorophyll to survive on photosynthesis alone, and thus is completely dependent on a host. By using a specialized organ called the haustoria, C. campesstris penetrates the host tissues and form connections with host vasculature to act as a strong sink. C. campesstris is a known agricultural pest, and infections often result in decreased yield and increased biotic stress. Small RNAs (sRNA) are known to be key regulators in post-transcriptional gene regulation and are used in a myriad of different processes of plant development and defense. MicroRNAs (miRNAs) are a class of sRNA which are single stranded hairpin derived molecules between 21-24nt long. Primary transcripts are processed by DCL1 to cleave the mature miRNA and miRNA* from the hairpin. The mature miRNA is then bound to AGO1 and is now capable of degrading mRNA transcripts which have complementarity to the miRNA sequence. Previously, our lab identified a population of parasite derived miRNAs which could move into host tissues and regulating gene expression involved in defense/hormone signaling. We termed these as interface-induced miRNAs (IIMs) as they are only detectable at the host/parasite interface. The previous work which identified these IIMs was done 10 days post attachment. To determine the day and phase of haustoria formation IIMs become detectable, a time course experiment was designed using both A. thaliana and S. lycopersicum as hosts. The time course begins once Cuscuta has coiled around a host, and the last time point is 14 days later. Interfaces were either subjected to RNA extraction and sRNA library construction or were subjected to vibratome sectioning and staining. sRNA sequencing was performed, and it was determined that all IIMs become detectable at the same time, day 2 in Arabidopsis and at day 1 in Tomato. There were no differences in the population of IIMs between the two different hosts. IIM are detectable early during the infection process. To

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identify which stage of haustoria formation IIMs become detectable, vibratome sectioning and staining with toluidine blue was performed following the time course described above. IIMs are detectable during the adhesive phase, which is before the parasite has even penetrated the host tissue. At the adhesive phase, the endophyte primordia are beginning to develop, and give rise to the fully developed haustoria. The conductive phase, which is the final phase of haustoria formation, is determined by a clear xylem bridge connecting the host and parasite vasculature. In both hosts, the conductive phase was identified 6 days after the parasite has coiled around the host. Furthermore, A 10nt cis-regulatory motif was identified upstream of 93% of the predicted IIMs. A literature review identified this as the upstream sequence element (USE) involved in snRNA transcription. All canonical miRNAs are transcribed by POL II, but current evidence suggests that IIMs are transcribed by POL III. If true, this would be the first recorded case of a miRNA being transcribed by POL III and yet remaining as a functional miRNA.

#39 **Examining the Relationship Between Per1 Expression in the Retrosplenial Cortex and Memory Formation**

D.J. Baldwin, C.A. Brunswick, K.K. Bodinayake, L. Bellfy, S.U. Murakami, J.L. Kwapis
Biology Department, Penn State University

Period 1 (Per1) is a circadian clock gene known for its role in the suprachiasmatic nucleus (SCN), where it maintains physiological circadian rhythms. Per1 has recently been implicated in another process independent of its role in the SCN: the gating of contextual memory formation within the dorsal hippocampus, a brain region integral to the formation of long-term contextual memory. Previous work has shown that upregulating Per1 within the hippocampus rescues the ability of aging mice to form contextual memories. Here, we sought to investigate the activity of Per1 within another memory-relevant structure, the retrosplenial cortex (RSC). The RSC is integral to the formation of long-term memory and is thought to bind complex stimuli together. To evaluate the impact of learning on Per1 expression, as well as the relationship between Per1 expression and memory performance, we trained mice at six timepoints throughout the circadian cycle using an object location memory (OLM) learning paradigm. We found that Per1 expression was induced by learning within the RSC in a circadian-dependent fashion, and that this induction oscillated in tandem with memory performance. Specifically, we found that both learning-induced Per1 and memory performance peaked during the day but were reduced at night. We also found that these circadian patterns of Per1 expression in the RSC change as a result of age. Although the magnitude of learning-induced Per1 expression was similar in young and old mice at each timepoint, the absolute levels of Per1 found in aging mice were lower across the board. This indicates that some critical level of retrosplenial Per1 might be necessary for the formation of long-term contextual memory, and that the epigenetic dysregulation associated with aging reduces Per1 expression below this threshold. Additionally, we show that while knockdown of Per1 within the RSC can impair a young mouse's ability to form long-term contextual memories, upregulation of Per1

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does not rescue memory impairments in aging mice. Together these results indicate that Per1 expression within the RSC may be necessary but not sufficient for the formation of long-term contextual memories. Future work would seek to expand our understanding of Per1's memory-gating function by identifying subsequent memory-relevant brain regions in which Per1 activity reflects what we have shown here.

#40 **Skin cell-derived Wnt ligands affect γ-tubulin localization in dendrites**

Pankajam Thyagarajan, Gregory Kothe, Melissa Long, Melissa Rolls
Biochemistry and Molecular Biology and the Huck Institutes of the Life Sciences, Penn State University

Local nucleation of microtubules at dendritic branchpoints is essential to maintain microtubule organization in Drosophila neurons. Canonical Wnt-signaling proteins including receptors and scaffolding proteins control the recruitment of the key player, γ-tubulin, to dendritic nucleation sites. Although Wnt-receptors Frizzled (fz), Frizzled-2, Ror and Arrow are all required for local nucleation at dendrite branch points, it is not yet known whether a Wnt ligand is required. Evi/Wntless is a carrier protein that is essential for Wnt-secretion in Wnt-producing cells. To identify the source of Wnt, we used Evi-RNAi as a tool to inhibit all Wnt secretion in specific cell types. We previously eliminated the possibility of autocrine Wnt signaling by knocking down Evi in neurons and finding no change in γ-tubulin localization. To test if paracrine Wnt signaling takes place - we considered neighboring cell types that are in proximity with the neurons: skin cells and glial cells. We constructed fly lines that enable knock-down of Evi specifically in the glial cells or the skin cells (UAS-gal4 expression system) while simultaneously visualizing γ-tubulin-GFP localization in neurons using a different expression system (QF-QUAS). The average branch point fluorescence of γ-tubulin-GFP in neurons (by in-vivo live-imaging) decreased with Evi-RNAi in skin cells but had no difference when Evi was depleted in glial cells. Epidermal knockdown of Porc, a transmembrane acyltransferase that causes lipidation of Wnt, also decreased γ-tubulin localization in neurons, suggesting skin cells as the source of Wnt. In addition, we tested the seven types of Wnt ligands present in flies to identify the specific skin cell-derived Wnt ligand involved. Surprisingly, knockdown of two Wnt ligands: Wnt-4 and Wnt-D in skin cells, decreased γ-tubulin localization in neurons. These data together identify epidermal cells as the source of two Wnt ligands that can impact γ-tubulin localization. We hypothesize that Wnt ligands are secreted by skin cells that surround sensory dendrites and interact with receptors at the dendrite surface. We had previously shown that nucleation at branch points occurs on endosomes containing Wnt receptors and scaffolds. To test whether these are locally generated by endocytosis at dendrite branch points, we examined clathrin localization in dendrites. Highly motile clathrin puncta are enriched at branch points. To test whether these were sites of active endocytosis we used a fast-acting temperature-sensitive allele of dynamin. After 15 minutes at the restrictive temperature, much fewer branchpoints with motile clathrin puncta were present. Together, these results are consistent with a model in which skin cells secrete

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Wnt ligands that are endocytosed at dendrite branch points to generate signaling endosomes that organize microtubule nucleation in dendrites.

#41 **MicroRNAs up-and-coming: Regulation of trans-species microRNAs biogenesis in Cuscuta campestris**

Ya-Chi Nien, Collin Hudzik, Michael J. Axtell
Biology Department, Penn State University

Dodders (Cuscuta spp.) are obligate parasitic plants that rob host plants of water and nutrient via an organ called the haustorium. We have found that C. campestris, the most agriculturally problematic species of dodder, sending novel microRNAs (miRNAs) into host plants through haustorium and shut down host messenger RNAs (mRNAs). One of the mRNAs, Sieve Element Occlusion Related 1 (SEOR1) helps seal the wound in phloem, and when silenced, seor1 mutants show increased exudation of sugars. It suggests that C. campestris evolved miRNAs to silence host SEOR1, thereby gaining more sugars by preventing phloem from clotting properly. These trans-species miRNAs must be under tight control to maximize parasitism success. Here, we explore the mechanisms govern trans-species miRNAs biogenesis and processing. On the promoter of all trans-species miRNAs, we discovered Upstream Sequence Element (USE) that is absent from canonical miRNAs. USE is proven to promote small nuclear RNAs (snRNAs) transcription by recruiting snRNA activating protein complex (SNAPc). Arabidopsis thaliana Shoot Redifferentiation 2 (SRD2) is one of the SNAPc subunits that is crucial for USE-dependent snRNA transcription. It is possible that trans-species miRNAs have co-opted pre-existing USE/SNAPc regulatory network to drive its transcription. To test this, constructs contain trans-species MIRNA loci with or without USE (USE+ / USE-), will be transformed into A. thaliana with either wild-type or srd2 background. If trans-species miRNA transcription is USE/SNAPc-dependent, reduced accumulation of trans-species miRNAs should be observed in both USE- and srd2 conditions. The USE will be directly deleted from C. campestris MIRNA loci to test USE-dependent transcription. To do this, CRISPR/Cas9-meditated genome editing and Host-Induced Gene Silencing (HIGS) will be combined into a new method called Host-Induced Genome Editing (HIGE). Cas9 protein along with gRNA targeting USE will be expressed in A. thaliana and translocated into C. campestris attached. Once translocated, the Cas9/gRNA complex will disrupt USE. The deletion of USE should reduce accumulation of trans-species miRNAs at HIGE interface if trans-species miRNA transcription is USE-dependent. After transcribed, canonical miRNAs are processed from their precursor (pre-miRNA) by Dicer-Like 1 protein (DCL1). To test if DCL1 is required for processing trans-species miRNAs, down-regulation of DCL1 in C. campestris (CcDCL1) will be achieved via HIGS, where homologous hairpin targeting CcDCL1 transferred from A. thaliana. Trans-species miRNA accumulation upon decreased CcDCL1 will be quantified by small RNA sequencing (sRNA-seq) and stem-loop qRT-PCR. Altogether, my research will (1) Test the hypothesis that the transcription of these dodder-derived, trans-species miRNAs is USE/SNAPc-dependent, and (2) Assess whether those

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transcripts rely on CcDCL1 to be processed. These experiments will directly test the importance of USE/SNAPc and CcDCL1 for trans-species miRNAs maturation without compromising to heterologous system and will be the first successful application of HIGS and HIGE with A. thaliana targeting Cuscuta spp.

#42 **Interactions between relative humidity and viral vector competence in Aedes aegypti**
Jaime Manzano, Gerard Terradas, Christopher J. Holmes, Sultan Asad, Joshua B. Benoit, Jason L. Rasgon
Entomology Department, Penn State University

According to the WHO, vector-borne diseases (VBDs) cause over 700.000 deaths annually. Aedes aegypti is a competent vector of multiple viral pathogens, including Dengue, Zika and Chikungunya viruses. Ae. aegypti evolved in Africa, but is now present in the Americas, Oceania, Asia, and Europe. The global distribution of this species and the pathogens it transmits are expected to change due to climate change. Relative humidity is one environmental variable that can drive the mosquito's hydration status and alter physiological and behavioral responses, such as bloodfeeding, which are relevant for pathogen transmission. Here, we exposed Aedes aegypti to different relative humidity treatments and challenged them with Mayaro virus to measure viral infection, dissemination, and transmission rates, as well as survival and bloodfeeding rates. Our results will help us understand the role of relative humidity in driving vector-borne disease dynamics.

#43 **The Effects of tRNA Methylation on Protein Synthesis in *Dictyostelium discoideum* - Research Updates**
Chau Duong, Denis A. Larochelle
Clark Univeristy, Worcester, MA

 One of the current standard therapies used for cancer treatment is epigenetic therapy. Epigenetics refers to any modifications affecting gene expression done without changing the genetic sequences, and these changes can be inherited. For example, attaching methyl groups to specific loci in DNA produces a chemical alteration that can block gene expression without the need for genetic mutation. This epigenetic inhibition activity can silence a tumor suppressor gene and promote tumor formation. Understanding the gene expression pathology is critical in cancer diagnosis and treatment. The role of epigenetic processes in gene regulation remains an area of active research. The field of epigenetics was initially defined with DNA (or **D**eoxyribo**N**ucleic **A**cid) methylation and histone modifications, while some other molecular targets in this process remained understudied. Besides DNA and histones, another target of epigenetic alterations is RNA (or **R**ibo**N**ucleic **A**cid). In recent years, RNA modifications have been shown to play critical roles in cancer physiology, which opened a novel research avenue for studying the role of epigenetics in cancer. One of the enzymes responsible for chemically modifying RNA through methylation in humans is Dmnt2 or Trdmt1, which was first identified as a DNA methyltransferase. It was later

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discovered that Dnmt2 could perform tRNA methylation on tRNA-Aspartate at Cytosine-38th position. In this research project, my focus is to study the tRNA methylation activity of **DnmA-** a homolog of human Dnmt2 in *Dictyostelium discoideum* (*D. discoideum*). Abnormal phenotypes of D. *discoideum* cells lacking the DnmA gene (DnmA KO strain) were observed. These included cells with altered cellular morphology, defects in cell division (cells with multinuclei and/or centrosomes, disorganized microtubules, or enlarged nuclei), and defects in development. Since Dnmt2 homologs are highly conserved among eukaryotes, including humans (*Homo sapiens*), fruit flies (*Drosophila melanogaster)*, fission yeasts (*Schizosaccharomyces pombe)*, and *D.discoideum*, it is predicted that DnmA plays important roles in cellular homeostasis across species. Similar to Dnmt2 activity, DnmA can also target tRNA instead of DNA for methylation. However, there is still a lack of connection between the specific tRNA methylation targets of DnmA and how the absence of this enzymatic activity can cause cell abnormalities in *D. discoideum*. To further understand this connection, in this research project, we will determine how tRNA methylation by DnmA affects protein synthesis and perform analysis of the potential downstream targets to identify the possible molecular pathways regulated by DnmA. The research project approach will utilize the recombinant DNA methods to generate DNA fluorescence reporter constructs, which can be transfected into *D.discoideum* wild-type and DmnA knock-out strains to examine the protein expression. RNA modifications through Dmnt2 remain a potential research area to develop different therapeutic targets for cancer treatment. By exploiting DmnA as the Dmnt2-homolog in *D. discoideum*, this research study will provide better understanding of the role of epigenetics via RNA modifications in gene expression and other cellular processes.

#44 **Characterization of disease progression in Alzheimer's disease mouse models by gender**
Mariel Micael, Ashley Brandebura, Nicola Allen
University of California San Diego, San Diego, CA

Astrocytes are a type of glial cell in the brain that regulates the formation, function, and elimination of neuronal synapses.In Alzheimer's Disease (AD), there is a loss of synapses in the hippocampus, making astrocytes a candidate cell type to target for therapeutic treatments aimed at slowing or preventing the loss of synapses. However, astrocytes are difficult to target in vivo with current genetic tools due to the availability of only a small number of specific markers. The Glial fibrillary acidic protein (Gfap) promoter is specific for astrocytes but Gfap is upregulated in Alzheimer's Disease, and is differentially expressed between wildtype (WT) and mutant (MUT) mice. We created a breeding scheme where Cre-dependent viruses can be targeted to astrocytes using ubiquitous promoters to drive the construct, but the specificity for astrocytes is achieved through Cre recombination driven by the astrocyte-specific promoter Aldh1L1. Aldh1L1 transcript is not differentially expressed between WT and MUT mice and thus is a preferable method to target astrocytes in AD. We crossed two common AD mouse models, APP/PS1 and Tau*P301S, to Aldh1L1-Cre. The APP/PS1 model exhibits amyloid pathology while the Tau*P301S model exhibits typical tau pathology, including neurofibrillary tangle (NFT) formation. The APP/PS1 and Tau*P301S mice have previously been characterized for the onset and progression of amyloid plaque and NFT pathology. However,

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given the complicated and multivariable nature of AD pathology and the fact that even small differences in the genome such as single nucleotide polymorphisms can alter the risk of developing disease in humans, we now want to characterize the time of onset and progression of gliosis, plaque and NFT pathology in the APP/PS1xAldh1L1-Cre and Tau*P301SxAldh1L1-Cre mice to determine if the onset and progression of disease is similar to uncrossed APP/PS1 and Tau*P301S mice. To study the gliosis and plaque formation in the APP/PS1xAldh1L1-Cre and Tau*P301SxAldh1L1-Cre mice, immunohistochemistry (IHC) will be used to visualize reactive astrocytes, plaques and tangles in the hippocampus and frontal cortex. WT and MUT mice of both genders will be characterized at 4 months, 6 months, and 9 months to compare disease onset and progression to published reports. Total area of Gfap will be used as a readout for the extent of astrocyte reactivity. The density, total number and average size of plaques and NFTs will be used as a readout for extent of amyloid and tau pathology. This data will ultimately help us as we move forward with testing Cre-dependent viruses targeting astrocytes to slow the onset and progression of AD.

Plant & Agricultural Sciences, Zoology, & Ecology

#10   **Role of the bovine PRAMEY protein in sperm function during in vitro fertilization (IVF)**
Chandlar Kern, Weiwei Wu, Chen Lu, Jianbin Zhang, Yaqi Zhao, Olga Maria Ocon-Grove, Peter Sutovsky, Francisco Diaz, Wan-Sheng Liu
Animal Science Department, Penn State

Preferentially expressed antigen in melanoma (PRAME) is a cancer/testis antigen (CTA) that is predominantly expressed in normal gametogenic tissues and a variety of tumors. PRAME proteins are enriched in the acrosome and sperm tail, but their role in sperm function is unknown. Our previous work revealed a decrease in the bovine PRAME, Y-linked (PRAMEY) protein in capacitated spermatozoa, and the release of the PRAMEY protein from the acrosome during the acrosome reaction, and therefore prompted the current study with the objective to examine the function of PRAMEY during fertilization. IVF was performed in multiple rounds using bovine matured oocytes and caudal epididymal sperm. Prior to IVF, spermatozoa were treated with PRAMEY antibody to determine the consequence of PRAMEY protein inhibition during fertilization. Normal rabbit IgG or Dulbecco's Phosphate-Buffered Saline (DPBS) were used as controls. For sperm-ZP binding analysis, oocytes were examined at 6 h post-IVF. A total of 59, 57, and 42 oocytes were evaluated for the PRAMEY antibody, rabbit IgG and DPBS treatments, respectively. The PRAMEY treatment group (34.44 sperm/oocyte) had nearly a 2-fold increase in the number of sperm bound to the zona when compared to both the rabbit IgG (17.57) and DPBS (18.07) controls (P<0.01). For the polyspermy evaluation, 148 and 134 fertilized eggs were examined at 45 h post-IVF for the PRAMEY antibody and rabbit IgG treatments, respectively. The polyspermy rate was 18.91%

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in the PRAMEY antibody-treated group, which was significantly greater than 5.97% observed in the rabbit IgG control (P<0.01). To confirm the polyspermy status in the fertilized eggs, we performed a MitoTracker™ green staining to label the mitochondria of spermatozoa prior to IVF. The results confirmed the presence of sperm tail with the mitochondrial sheath within the fertilized egg after 16 h of fertilization. In addition to sperm binding and polyspermy analysis, we also studied early embryo development by counting the number of embryos with 1-, 2-, ≥4-cells at 45 h post-IVF. We found that the percentage of 1-cell embryos in the PRAMEY antibody treatment (55.38%) tended to be lower than that in the rabbit IgG control (64.57%) (P>0.05), while percentage of 2-cell embryos was very similar between the PRAMEY antibody (25.10%) and rabbit IgG treatments (26.46%) (P>0.05). However, the percentage of ≥4-cell embryos in the PRAMEY antibody-treated group (19.52%) was two-fold greater than that in the rabbit IgG control (8.97%) (P=0.0026). Additionally, 82 and 89 embryos, fertilized by spermatozoa treated with PRAMEY antibody or rabbit IgG during capacitation were evaluated at 45 h post IVF. The percentages of 1-cell, 2-cell, and 4-cell embryos were similar no matter if spermatozoa were treated with PRAMEY antibody or rabbit IgG during capacitation, suggesting that inhibition of PRAMEY during capacitation had no impact on IVF. In summary, our results indicated PRAMEY's potential involvement in anti-polyspermy defense and early embryo cleavage. This research provides the initial evidence for the involvement of the PRAME protein family in sperm function, fertilization, and early embryo development.

#13 **Does milkweed species influence monarch survival? Maximizing conservation efforts of the monarch butterfly in the face of predators.**
Lillian Germeroth, Sophia Mucciolo, Sara Hermann
Ecology IDGP, Penn State University

Monarch butterflies are a poster child for insect conservation due to their charisma and population decline over the past 20 years. The decline of this beloved species has been met with a desire from the public to be involved with conservation action. However, the approach to conserving this migratory species is complex, and their decline is multi-factorial; prior efforts have primarily focused on mitigating the loss of milkweed hostplants from the summer breeding landscape which is largely attributed to agricultural development. Since conservation plantings often incorporate several species of milkweed, the question becomes which species combinations best ensure monarch survival? Milkweed species vary in their physical traits as well as the predatory arthropods they harbor. Monarchs face a steep population bottleneck in the first days of development that is thought to be driven by predation. The aim of this work is to catalog the arthropod communities of the three most commonly planted milkweed species in the Northeastern US (Asclepias syriaca (common milkweed), A. incarnata (swamp milkweed) and A. tuberosa (butterfly milkweed)), investigate the interactive effects of plant species on predator community, and the significance of these factors on monarch egg and larval survival. To assess this, we surveyed

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10 gardens in the State College area that had established populations of each of the milkweed species in question for 13 weeks June to September 2021. We found a dramatic difference in arthropod communities between milkweed species, driven not only by variance in predator presence and abundance, but by the diversity and abundance of herbivorous aphids which have indirect negative effects on monarch oviposition and survival. Future manipulative experiments will elucidate the causative relationships between these community differences and monarch success. These findings will inform and maximize conservation efforts of monarchs in the face of invertebrate predators and provide gardeners with practical tools to contribute to arthropod conservation in their own backyards.

#16 **The mouse Pramel1 gene regulates spermatogonial development through the retinoic acid (RA) signaling pathway**

Mingyao Yang, Wenzhi Ma, Jon Oatley, Wansheng Liu
1 Department of Animal Science, Center for Reproductive Biology and Health (CRBH), College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA and Center for Reproductive Biology, School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

The PRAME protein, a cancer/testis antigen (CTA) and a nuclear receptor transcriptional regulator, acts as a repressor of RA receptor (RAR) to inhibit cancer cell differentiation. However, it is largely unknown the function of PRAME family proteins in germ cells. To test the hypothesis that PRAMEL1 is involved in RAR signaling in spermatogenic cells, we have generated and characterized two models of the Pramel1 knockout (KO) mice. One was a global KO (gKO) by a CRISPR/Cas9 approach, and another was a conditional KO (cKO) by Cre-loxP with the Stra8-Cre. We found that the gKO mice had some distinct phenotypes comparing with cKO which were related to the germ cell's response to RA in the neonatal testis. No difference was observed in the testis weight/index and the number of prospermatogonia among the wild-type (WT), gKO and cKO mice at postnatal day 2 (P2). However, from P3 onward, we observed two significant phenotypes among these juvenile animals. First, the testis weight/index were significantly increased in the juvenal gKO mice (P14) ($P<0.01$) comparing to WT and cKO mice. Meanwhile, the gKO males produced 38.4% more sperm than WT and cKO males at P41, which leads to a significant increase in litter size ($P<0.01$) to make the gKO to be superfertile. Second, approximately 7% seminiferous tubules were abnormal with some germ cell loss in both gKO and cKO mice compared to WT. Remarkably, these abnormal tubules showed a Sertoli cell-only (SCO) phenotype in the gKO but not in cKO mice during the first round of spermatogenesis. To further determine how Pramel1 is involved in spermatogonia response to RA at P3, we performed immunofluorescence (IF) staining with germ (PLZF, TRA98, and STRA8) and Sertoli (SOX9) cell makers on neonatal testis cross-sections which indicated that the number of undifferentiated (PLZF+) spermatogonia was not affected in the Pramel1 gKO mice at P2 ($P>0.05$), but decreased at P3-P6 ($P<0.01$) when germ cells respond to the first peak of RA

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pulse. At the same time, the differentiating (PLZF+STRA8+) spermatogonia were increased in the gKO mice (P<0.01). We applied TUNEL assay to study germ cell apoptosis from P7 to P35 testis and found that WT mice had a normal curve of apoptotic germ cells that peaks around P14. However, the peak was not observed in the gKO mice. Increased differentiating spermatogonia and less apoptosis explain why the gKO mice produced more sperm during the first round of spermatogenesis. To explain how SCO tubules were formed in gKO but not in cKO mice, we isolated seminiferous tubules at P2-P6 and performed the whole-mount dual-staining with TRA98 & SOX9 and TRA98 & CASP3 (cell apoptotic marker). We found that SCO regions were originated in the Pramel1 gKO testis from P3 onward. Germ cells at either end of the SCO region failed in homing and underwent apoptosis at P3. Together, our data indicated that deletion of Pramel1 before and after P3 would lead to different phenotypes during the first round of spermatogenesis, suggesting the involvement of PRAMEL1 in the RAR signaling pathway.

#17 **The effect of intrauterine infusions of IFNT and PAG on the expression of early pregnancy factor (HSPE1).**
Ty Montgomery, M. Isabel da Silva, Joy Pate, and Troy Ott
Animal Science, Penn State University

Pregnancy success depends on communication between the embryo and maternal endometrium. In cattle, one of the earliest transcribed embryo-derived proteins is a heat shock protein family E member 1 (HSPE1). Originally termed early pregnancy factor, HSPE1 is produced by the ovary and present in blood serum 6 to 24 hours after mating. It is continuously synthesized throughout early pregnancy by the ovary and oviducts. Furthermore, embryos fail to attach when the mother is immunized with anti-HSPE1 antibodies. In cattle, interferon tau (IFNT) is a primary conceptus-derived protein (days 8 to 30) known to induce corpus luteum maintenance, a requirement for pregnancy success. IFNT regulates gene expression in the endometrium and peripheral blood leukocytes (PBL). Shortly after the onset of IFNT production, the conceptus secretes pregnancy associated glycoproteins (PAG; day 17 to calving) in high concentrations. However, the function of PAG remains unknown. We determined the effects of IFNT and PAG on HSPE1 transcription in the endometrium and PBL. Holstein heifers (n=6/treatment) were estrous (day 0) synchronized and received twice daily intrauterine infusions of 20 mL of saline including one of the following treatments: vehicle 200 μg/mL BSA from day 14 to 16 (BSA3), vehicle + 200 μg of IFNT from day 14 to 16 (IFNT3), vehicle + 200 μg of IFNT from day 14 to 19 (IFNT6), and IFNT3 from day 14 to 16 followed by IFNT3 + 100 μg pregnancy specific protein B (PSPB; a mixture of PAG isolated from the bovine placenta) from day 17 to 19 (PSPB6). Blood was collected daily, and an endometrial biopsy was obtained at the end of treatments. RNA was extracted from PBL and endometrium, and real-time quantitative polymerase chain reaction was used to quantify HSPE1 expression. Data were analyzed using the MIXED procedure in SAS, and the model tested the effects of treatment, day, and their interaction. In PBL, there was no effect of

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treatment, however, an effect of day (P<0.02) was detected for HSPE1 expression in BSA3 and IFNT3 treatments. Regression analysis confirmed that HSPE1 transcription increased from day 14 to day 15 and then declined by day 17 (Day, quadratic, P<0.004). There was no effect of treatment on HSPE1 expression in the endometrium collected after 3 days of treatment. However, a treatment by day interaction (P<0.01) on HSPE1 expression was detected in PBLs between IFNT6 and PSPB6 treatments. HSPE1 transcription increased in PBLs after from day 18 to 20 in the IFNT6 group but there was no change in HSPE1 expression in the PSPB6 group. There was no effect of treatment on HSPE1 expression in the endometrium. Results suggest that PSPB alters the effects of IFNT on HSPE1 expression in PBL but not endometrium. A better understanding of the effects of conceptus proteins on gene expression in the uterus and in PBL should help elucidate factors affecting fertility. This work is supported in part by AFRI competitive grant 2017-67015-26455 from USDA-NIFA to TLO.

#22 **Impact of superheated steam roasting on the polyphenol composition, bioactivity and volatile compound profile of cocoa beans**

Sawali Navare, Ramaswamy Anantheswaran, Joshua Lambert
Food Science Department, Penn State University

Cocoa beans are a rich source of polyphenolic compounds with anti-inflammatory and enzyme inhibitory activities which could aid in obesity management. Cocoa processing steps like hot air roasting have been shown to reduce the total polyphenol content (TPC) by more than 50%. Superheated steam roasting has been reported to retain higher TPC levels in cocoa as compared to hot air roasting. Changes in TPC might not be indicative of changes in bioactivity of these cocoas. Some studies have shown that the digestive enzyme inhibitory and anti-inflammatory properties of cocoa remain intact under certain processing conditions irrespective of TPC loss. This suggested that the composition of polyphenols rather than the total amount may be a more important determinant of bioactivity. Hot air roasting at three temperatures (150C, 175C, 200C) was compared to superheated steam roasting in terms of its impact on the polyphenol composition, bioactivity, and flavor development in cocoa. Cocoa beans roasted using these two methods were analyzed for TPC using Folin-Ciocalteau assay. The inhibitory effect of cocoas against Phospholipase A2 was determined. TPC of hot air and superheated steam roasted beans was not significantly different (α=0.05). However, the IC50 of cocoa extracts obtained from superheated steam roasted beans at 175C against Phospholipase A2 was about 62% lower (α=0.05) than that of extracts from hot air roasted beans, indicating higher bioactivity of superheated steam roasted beans. The volatile compound profiles of the cocoa beans roasted by both methods were compared using GC-MS. Cocoa beans roasted for 30 min at 150C with superheated steam exhibited 30-50% higher levels of desirable volatile compounds like pyrazines as compared to hot air roasted beans. The procyanidin composition of beans prepared by these different roasting methods is being

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compared using LC-MS to offer insight into the higher bioactivity of superheated steam roasted cocoa.

#25 **Characterization of Cd transport activity of candidate HMA genes from T. cacao**
Francisco Menendez, Mark Guiltinan, Siela Maximova
Plant Sciences Department, Penn State University

The seeds of Theobroma cacao, the main ingredient in chocolate, can accumulate toxins such as the harmful heavy metal cadmium (Cd). This has prompted regulations that limit the amount of Cd allowed in cacao derived products as well as research on mitigation solutions. Cd uptake and accumulation share many molecular and physiological pathways used by plants to manage other elements. This work investigates one of the main families of Zinc (Zn) and Cd transporters in plants, the Heavy Metal ATPases (HMA). These play an important role in Cd redistribution and storage in many plant species. Coding sequences from cacao were identified and their evolution compared to other plant species. Natural variation of the HMA genes was investigated in 38 sequenced cacao genomes. A candidate HMA2/3 gene from cacao was demonstrated to have Cd transport capacity in yeast.

#29 **Endometrial transcriptome in response to intrauterine infusions of secretory proteins from the bovine conceptus**
M. Isabel da Silva, Ty Montgomery, Joy Pate and Troy Ott
Animal Sciences Department, Penn State University

Pregnancy involves an orchestrated communication between the embryo and endometrium. Within the first 25 days of pregnancy, embryonic secretions are crucial for maternal recognition of pregnancy. In cattle, interferon tau (IFNT) is a primary embryonic signal (days 8 to 30) known to support corpus luteum maintenance, a requirement for pregnancy success. Shortly after the onset of IFNT production, the conceptus secretes pregnancy associated glycoproteins (PAG) (day 17 to calving) in high concentrations. However, the function of PAG remains unknown. This study reproduced aspects of the temporal hormone signaling during early pregnancy to study the short and long-term effects of IFNT and PAG on the endometrial transcriptome. Holstein heifers (N=4/treatment) were estrous synchronized and received twice daily intrauterine infusions of 20 mL of saline including one of the following treatments: vehicle 200 μg/mL bovine serum albumin (BSA) from day 14 to 16 (trt-BSA3), vehicle + 200 μg of IFNT from day 14 to 16 (trt-IFNT3), vehicle + 200 μg of IFNT from day 14 to 19 (trt-IFNT6), and trt-IFNT3 from day 14 to 16 followed by trt-IFNT3 + 100 μg pregnancy specific protein B (PSPB; a mixture of PAG isolated from the bovine placenta) from day 17 to 19 (trt-PSPB6). Blood was collected daily, and endometrial biopsies were obtained at the end of treatments for RNA extraction. Transcriptomic data were obtained through BGI (Shenzhen, China) services. Differentially expressed genes (DEGs) were considered significant if $log2 \geq 1$ and Q-value < 0.15. All treated animals had plasma

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progesterone concentrations that were comparable to pregnant heifers. Compared to trt-BSA3, trt-IFNT3 upregulated 901 and downregulated 777 DEGs, mostly involved in interferon and innate immune signaling. Compared to trt-IFNT3, trt-IFNT6 upregulated 16 and downregulated 25 DEGs, involved in glycogen and tryptophan metabolism, progesterone and retinoic acid signaling, and antigen recognition in natural killer cells. Compared to trt-IFNT6, trt-PSPB6 upregulated 4 transcripts related to mucus secretion (MUC5AC) and inhibition of extracellular proteinase (WFDC18) and peptidase (EXPI). Our results show signaling pathways in the endometrium that may be directly or indirectly regulated by IFNT and PSPB during early pregnancy. Moreover, we identified transcriptomic changes during prolonged exposure to IFNT and genes regulated by PSPB in the endometrium. Results provide a greater understanding of the effects of conceptus secretions on uterine function during a period of high embryo loss. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26455 from the USDA, National Institute of Food and Agriculture.

#33 **Dynamic Regulation of the Stability of Petunia S-locus F-box (SLF) Proteins Involved in Pollen Function of Self-Incompatibility**

Linhan Sun, Penglin Sun, Lihua Wu, Patrick Stone and Teh-hui Kao
BMMB Department, Penn State University

Self-incompatibility in Petunia inflata is controlled by the polymorphic S-locus, which contains S-RNase determining pistil specificity and, for S2- and S3-haplotypes, 17 S-locus F-box (SLF) genes for pollen specificity. All SLF proteins of a given S-haplotype collectively mediate ubiquitination and degradation of their non-self S-RNases, but not self-S-RNase, in the pollen tube cytosol resulting in cross-compatible, but self-incompatible, pollination. Ubiquitination of S-RNases requires that each SLF be assembled into an SCF (Skp1–Cullin1–F-box) complex, which also contains pollen-specific Skp1-like protein (PiSSK1) and Cullin1 (PiCUL1-P), and a conventional RBX1 protein (PiRBX1). Previously, we reported that SLF proteins were themselves subject to ubiquitin–26S proteasome mediated degradation in vivo. To further examine regulation of SLF stability in pollen, we separately introduced S2-SLF1:GFP and S2-SLF8:GFP transgenes (driven by a pollen-specific promoter, LAT52) into plants whose production of PiSSK1 had been knocked out using CRISPR/Cas9. In the absence of PiSSK1, GFP fluorescence and protein levels of S2-SLF1:GFP and S2-SLF8:GFP in pollen tubes were reduced dramatically, whereas the GFP fluorescence and protein level of S2-SLF1:GFP with an 18-amino-acid degron deleted (S2-SLF1-Δ295-312:GFP) remained unaltered. The transcript levels of S2-SLF1:GFP and S2-SLF8:GFP were similar to those in PiSSK1 wild-type background. These results suggest that the absence of PiSSK1 in pollen results in degradation of SLF proteins by the 26S proteasome pathway. Using co-immunoprecipitation followed by mass spectrometry, we found that an SCF-complex-dissociation protein, PiCAND1, and a HECT-type E3 ligase, PiUPL1, co-immunoprecipitated with S2-SLF1:GFP in the presence of a 26S proteasome inhibitor, MG132. We further

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confirmed that PiCAND1 interacts with PiCUL1-P. We propose a model for the dynamics of SLF proteins in vivo, in which the assembly of SLF proteins into SCFSLF complexes stabilizes them, and PiCAND1-mediated dissociation of the SCFSLF complexes not needed, or no longer needed, for detoxifying specific non-self S-RNases produced in the style, exposes SLF proteins to PiUPL1 for degradation.