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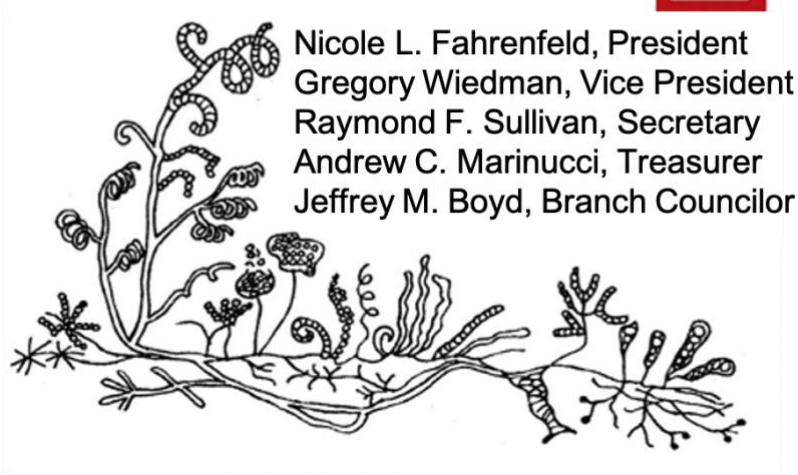

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Spring 2023 Symposium Proceedings

19 May 2023

Richard Weeks Hall of Engineering

Rutgers University, Piscataway, NJ

Opening remarks and presentation of awards by Nicole Fahrenfeld, President, Theobald Smith Society Nicole serves as the 81th president of the Theobald Smith Society. She is associate professor of Civil & Environmental Engineering in the School of Engineering, Rutgers University, Piscataway, NJ. Fahrenfeld earned her PhD and completed her post-doctoral research at Virginia Tech, Blacksburg, VA. Her primary research interest lies in at the interface of environmental chemistry and environmental microbiology to promote water quality and sustainability, with applications in natural, agricultural, and engineered systems. Research interests / projects include microbial source tracking in mixed land use watersheds, end-of-pipe treatment for combined sewer overflows, microbial processes in sewers, microplastic sources and their associated biofilms, antibiotic resistance monitoring in urban waters and bioremediation of oil, solvent, and explosives contaminated aquifer sediments.

Invited Speakers

Jennifer Sun, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ The effect of endosymbionts on insect host olfactory behavior.

Grace Beggs, Department of Molecular Biology, Princeton University, Princeton, NJ A novel phage anti-repressor enables phage VP882 to eavesdrop on *Vibrio cholerae* quorum-sensing-communication.

Dhanya Dhanyalayam, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ Male susceptibility and Female resilience: Understanding COVID-19 mortality in mice.

Nishant Sharma, Public Health Research Institute, Department of Medicine, New Jersey Medical School, Rutgers University, Newark, NJ Role of dynamin-like proteins in extracellular vesicles secretion by *Mycobacterium tuberculosis*.

Alanna Cohen, Department of Plant Biology, Rutgers, the State University of New Jersey, New Brunswick, NJ Factors driving genome evolution of *Anisogramma anomala*, the Eastern Filbert Blight fungus, reveal lifestyle and pathogen biology.

Jisun Kim, Department of Pathology, Immunology and Laboratory Medicine, Center for Inflammation and Immunity, Rutgers New Jersey Medical School, Newark, NJ Oxidative phosphorylation potentiates antimicrobial sensitivity in *Staphylococcus aureus*.

Young Investigator Award Presentation: Dana Price is Associate Research Professor in the Department of Entomology, School of Environmental & Biological Sciences, Rutgers University. His research focuses on functional genomic analyses of vector arthropods and their holobiont - that is, the host and assemblage of commensal organisms (viruses, bacteria and eukaryotes) that live within and around them. His current research initiatives include: novel means of rapid and non-destructive vector-borne pathogen sampling and discovery, reverse-vaccine development targeting invasive arthropods, tick-borne disease dynamics and discovery in a rapidly changing landscape, and genotyping and spatiotemporal distribution of arthropod-borne viruses.

2023 Waksman Award Lecture by Zemer Gitai: Bugs to Drugs: From Bacterial Cell Biology to Novel Antibiotics The rise in resistance to known antibiotics has made developing new approaches to combatting bacterial pathogens increasingly urgent. I will discuss work from my lab that aims to address this unmet need by bringing quantitative and biophysical perspectives to the problem. For example, we have combined machine learning and morphometric studies to design screens for antibiotics with novel mechanisms of action. Meanwhile, novel methods for single-cell transcriptional analysis enable new insights into the heterogeneity of bacterial responses to antibiotics, which may in turn help improve their use in treating infections. All of these efforts are based on the foundation of bacterial cell biology, which enables us to understand how bacterial grow and interact with their environments at a quantitative and single-cell level.

Zemer Gitai is the Edwin Grant Conklin Professor in the Department of Molecular Biology at Princeton University. His research focuses on the cell biology of bacteria. His lab studies how cells self-organize across spatial scales, using quantitative, molecular, and engineering

approaches to understand problems such as cell shape formation, host-pathogen interactions, and antibiotic development. His work discovered new components of the bacterial cytoskeleton, new functions for bacterial polymers in metabolism, compartmentalization, and chromosome dynamics, and established the importance of protein assembly for unexpected processes like metabolism and pathogenesis. More recently, the Gitai lab has extended its use of quantitative methods to discover novel features of microbe-host interactions and antibiotics with novel mechanisms of action. Gitai's achievements have been recognized by awards such as the NIH Director's Pioneer Award, the NIH New Innovator Award, the NIH Transformative Research Award, the Beckman Young Investigator Award, and the Human Frontier Science Program Young Investigator Award.

Poster presentation award winners

Grace Beggs, Department of Molecular Biology, Princeton University, Princeton, NJ A novel phage anti-repressor enables phage VP882 to eavesdrop on *Vibrio cholerae* quorum-sensing-communication. (Abstract #4)

Joshua Chamberlain, Center for Computational and Integrative Biology, Rutgers University, Camden, NJ and Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ Effects of bacterial sphingolipids on the properties of synthetic liposomes. (Abstract #5)

Geordan J. Stukey, Dept. of Food Science and Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ Phosphatidate phosphatase Pah1 contains an inhibitory domain that regulates its function in yeast lipid synthesis. (Abstract #13)

Alina Thokkadam, Dept. of Chemical and Biological Engineering, Princeton University, Princeton, NJ Evaluation of structure-activity function of a lasso peptide using high-throughput screening. (Abstract #15)

Brian Choi, Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ Unique enzymatic aspartimidylation of omega-ester-containing peptides in *Actinomycetota*. (Abstract #19)

POSTERS

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1. A novel strategy to improve microbial biosynthesis of aromatic products through global metabolism regulation.

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Aromatics are an essential group of compounds with recognized values in the chemical, pharmaceutical, and food industries. Biosynthesis of aromatics from renewable feedstocks has been achieved through utilizing the shikimate pathway in engineered microbes. However, the low bioproduction yield resulting from the inherent competition between cell growth and biosynthesis is a major challenge for the broad application of this technology. In this study, we developed a novel strategy to enhance the biosynthesis of shikimate pathway derivatives by utilizing an antimicrobial peptide to block protein synthesis and repress global metabolism in selected microbial host *E. coli*. We confirmed that this strategy worked to direct more metabolic resources toward the biosynthesis of different aromatic products, including 4-hydroxybenzoate, tyrosine, and phenol. Through rational manipulation and optimization, we significantly improved the yield of these desired products. This study demonstrates the effectiveness and general applicability of this strategy for the bioproduction of various aromatic derivatives, providing a promising perspective to advance metabolic engineering.

2. Immune response alterations caused by mechanical substrate stiffness within a UTI model.

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1. Center for Computational & Integrative Biology, Rutgers University, Camden, NJ
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3. Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ

Urinary tract infections escalate healthcare burdens and have been increasing over the past 20 years. How the immune system responds to these infections is incompletely understood especially as it relates to the induction of host cell signaling pathways. This is crucial because it has been shown that uropathogenic *E. coli* (UPEC) elicits an inflammatory response during mouse infections but has a limited effect during cell culture infections of bladder epithelial cells. In an effort to understand this discrepancy, and to study the signal transduction pathways, we are considering the role of tissue stiffness in regulating the inflammatory response. We have previously demonstrated that the application of physiologically soft polyacrylamide gels aids in the recapitulation of the internal bacterial lifecycle within immortalized bladder epithelial cells. We now show that these gels also affect the timing of the production of cytokines such as IL-6, IL-8, & TNF during infection with UPEC. This result is surprising as the use of a passive soft substrate typically lowers the direct production of these and other cytokines. We are continuing to study the mechanosensitive signal transduction pathways involved in regulating the bladder cell inflammatory response to UPEC.

3. The effect of endosymbionts on insect host olfactory behavior.

Muqaddasa Tariq, Safiyah Salama, Hazem Al Darwish, Jennifer S Sun

Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ

Insects rely heavily on their sense of smell to identify food, mates, and predators, and to ensure their survival. This insect olfactory system is plastic, adapting to external and internal cues to enable the insect to respond to its environment. Of particular interest is the effect that endosymbionts, the essential, mutually-beneficial bacteria which live within insects, have on insect olfactory-guided behaviors. Insect gut microbiota and their associated enzymes have been shown to play pivotal roles in detoxification, immunity, and assist with deriving nutrients from food. These symbiotic gut bacteria can even influence host development and ecological interactions. However, not much is known about whether bacteria are present in insect tissues aside from the gut, and whether those bacteria are also capable of producing physiological changes in the insect. We first reveal the identity of endosymbionts in the pervasive mosquito vector *Aedes albopictus*. Then, in the genetically-tractable model organism *Drosophila melanogaster*, which has comparable tissue composition and established behavioral paradigms, we determine the impact of each endosymbiont on global insect physiology and olfactory-guided behaviors. By doing so, this study reveals how gut bacterial communities dictate the success of vector-host interactions, which is a significant aspect of pathogen transmission. Our discoveries may lead to the development of new insect repellents that mimic, augment, or counteract endosymbiont-produced factors, which are safe, inexpensive, and environmentally friendly. The study may also provide better insight into the mechanism of olfaction across species, through the study of a highly evolutionarily conserved interspecies relationship.

4. A novel phage anti-repressor enables phage VP882 to eavesdrop on *Vibrio cholerae* quorum-sensing-communication.

Grace A. Beggs[1], Bonnie L. Bassler[1,2]

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Bacteria are bombarded by infecting viruses, called phages, in natural habitats. Upon infection of a host bacterium, temperate phages must undertake one of two lifestyles: lysogeny or lysis. During lysogeny, the phage remains in the host and is passed down to offspring. When the phage switches to the lytic program, it replicates, kills the host, and spreads to new cells. Phages have been thought to transition between lysogeny and lysis exclusively in response to host stress and DNA damage. New research has upended this dogma by revealing that phages can monitor host sensory cues and exploit the information they garner to drive lysogeny-lysis lifestyle transitions. Indeed, some phages can surveil bacterial quorum-sensing communication molecules to tune their lysis program to high host cell density, a strategy that presumably maximizes transmission to new hosts. I am using a combination of genetic, biochemical, and structural approaches to explore mechanisms underlying chemical communication between vibriophage VP882 and its host, the global pathogen *Vibrio cholerae*. Phage VP882 possesses an anti-repressor that has no known structural homologs and is critical for launching the quorum-sensing-induced lysis pathway. My initial biochemical studies reveal that this anti-repressor, comprised of only 79 amino acids, forms an SDS-resistant tetradecamer, and oligomerization may be crucial for its biological function. Recently, the Bassler group discovered additional phage anti-repressors that function analogously to the one from phage VP882. Thus, this work could reveal the central mechanisms underlying a new class of phage-encoded proteins.

5. Effects of bacterial sphingolipids on the properties of synthetic liposomes.

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The research field of drug delivery has been pursuing liposomal drug delivery systems for nearly 30 years. These liposomes and their components, including lipids, must be highly characterized to provide the ideal conditions for effective drug delivery. Some key features include membrane permeability, fluidity, and stability. Among the commonly used lipids in liposome formation are ceramides and sphingolipids. Recent discoveries regarding bacterial ceramide synthesis have created opportunities to incorporate novel, previously uncharacterized, bacterially derived sphingolipids into these synthetic membranes. Here, we report on the characterization of the bacterial sphingolipids and ceramides native to the *Caulobacter crescentus* lipidome and quantify their effects on membrane permeability and fluidity.

6. Macrophages with depleted mtDNA have depressed immune response.

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Mycobacterium tuberculosis (Mtb), the intracellular bacterial pathogen that causes tuberculosis (TB), remains one of the deadliest infectious diseases worldwide and killed 1.6 million people in 2021. Mtb evades killing in the macrophage by secreting bacterial proteins into the host cytosol that promote its own survival and replication. However, in doing so, Mtb also releases DNA into the cytosol, which is sensed by the macrophage via the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS). Upon binding DNA, cGAS activates the STING/TBK1/IRF3 signaling axis, which causes an immune response marked by increased expression of interferon-β (IFNβ). IFNβ is a type I interferon (IFN) traditionally associated with antiviral defense mechanisms, but this antiviral response is often detrimental during bacterial infections. Type I IFNs inhibit the activity of critical antibacterial cytokines like IFNγ and IL-1β, both of which are essential for responding to bacterial pathogens like Mtb. Previous work has shown that in addition to detecting cytosolic bacterial DNA, cGAS can detect other types of host DNA such as cytosolic mitochondrial DNA (mtDNA); therefore, the precise origin of cGAS-bound DNA during Mtb infection remains unclear. To better understand the respective contribution of host and bacterial DNA, we are assessing how the depletion of mtDNA in macrophages (RAW 264.7, immortalized bone marrow-derived macrophages (iBMDM), and U937 macrophage cell lines), affects activation of the cGAS/STING/TBK1 signaling axis during Mtb infection. We depleted macrophage mtDNA by growing cells in the presence of 2′-3′-dideoxycytidine (ddC), which preferentially inhibits DNA replication in mitochondria, thus diluting their mtDNA as the cells grow and divide. We analyzed the mtDNA content of ddC-treated cells via qPCR and found they had less than 2% of their initial mtDNA content. To measure the baseline type I IFN responses of mtDNA-depleted macrophages, we stimulated them with cytosolic double-stranded DNA and measured type I IFN induction. Both control and mtDNA-depleted macrophages had about 3,000-fold induction of IFNβ and 30-fold induction of IRF7 upon stimulation, demonstrating that depleting mtDNA in macrophages does not inherently affect this signaling axis. Future work will use these mtDNA-depleted macrophage cell lines to investigate their type I IFN expression in the context of bacterial infection, where mtDNA has the potential to contribute as a cGAS ligand. Ultimately, we will use these studies to define the source of the cGAS-activating DNA during Mtb infection, where we predict that both bacterial DNA and mtDNA contribute, possibly with differing dynamics and magnitude. Together these results will inform the mechanism by which the STING/TBK1/IRF3 axis is activated during Mtb infection and reveal novel host targets for future therapies to treat Mtb infection.

7. Nε-Lysine acetylation of the histone-like protein HBsu regulates the process of sporulation and affects the resistance properties of *Bacillus subtilis* spores.

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Bacillus subtilis produces dormant, highly resistant endospores in response to extreme environmental stresses or starvation. These spores are capable of persisting in harsh environments for many years, even decades, without essential nutrients. Part of the reason that these spores can survive such extreme conditions is because their chromosomal DNA is well protected from environmental insults. The α/β-type small acid soluble proteins (SASPs) coat the spore chromosome, which leads to condensation and protection from such insults. The histone-like protein HBsu has been implicated in the packaging of the spore chromosome and is believed to be important in modulating SASP-mediated alterations to the DNA, including supercoiling and stiffness. Previously, we demonstrated that HBsu is acetylated at seven lysine residues, and one physiological function of acetylation is to regulate chromosomal compaction. Here, we investigate if the process of sporulation or the resistance properties of mature spores are influenced by the acetylation state of HBsu. Using our collection of point mutations that mimic the acetylated and unacetylated forms of HBsu, we first determined if acetylation affects the process of sporulation, by determining the overall sporulation frequencies. We found that specific mutations led to decreases in sporulation frequency, suggesting that acetylation of HBsu at some sites, but not all, is required to regulate the process of sporulation. Next, we determined if the spores produced from the mutant strains were more susceptible to heat, ultraviolet (UV) radiation and formaldehyde exposure. We again found that altering acetylation at specific sites led to less resistance to these stresses, suggesting that proper HBsu acetylation is important for chromosomal packaging and protection in the mature spore. Interestingly, the specific acetylation patterns were different for the sporulation process and resistance properties of spores, which is consistent with the notion that a histone-like code exists in bacteria. We propose that specific acetylation patterns of HBsu are required to ensure proper chromosomal arrangement, packaging, and protection during the process of sporulation.

8. Interrogating the gut microbiota of colorectal cancer patients.

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths in the US. Every year, ~150,000 new CRC cases are reported. Several factors can increase the risk for CRC, i.e., genetics, lifestyle, and the gut microbiota. While human genetics and lifestyle have been extensively studied for their role in CRC development, the role of the gut microbiota is still unclear. Therefore, this project aims to interrogate the role of the gut microbiota and its small molecule products in CRC development. First, I will perform a meta-analysis of metagenomic data of CRC cohorts and their matched controls to identify CRC-associated small molecules biosynthetic gene clusters. Then, I will utilize in vitro and in vivo screening assays to prioritize and test the role of specific microbiota members in CRC development. The small molecule products of the prioritized gut microbiota members will be pursued to establish possible causal links. These experiments will highlight the potential role of the gut microbiota in CRC development and will inform future endeavors to target exact microbiota members for developing new therapies.

9. Male susceptibility and female resilience: understanding COVID-19 mortality in mice.

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COVID-19, caused by SARS-CoV-2, primarily affects the respiratory system, but severe cases may lead to extra-pulmonary symptoms. Men who contract the disease tend to experience more severe symptoms and higher mortality rates than women. Previous studies have shown that the virus can reside in adipose tissue. In this study, we investigated the role of adipose tissue in SARS-CoV-2 infection and pulmonary pathology in male and female humanized ACE2 mice. Our results showed that females had higher survival rates than males, and while males had higher viral loads in their lungs, females had higher viral loads in their adipose tissue. We found an inverse correlation between the viral loads in the lungs and adipose tissue. In addition, we showed that male mice experienced more severe pulmonary pathology than female mice when infected with CoV2. Therefore, our data suggest that in female mice, adipose tissue may act as a reservoir for the virus, reducing the viral load in the lungs and preventing lung damage due to immune cell infiltration and pro-inflammatory cytokines.

10. Understanding how post-antibiotic events impact *Escherichia coli* bacterial persistence.

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Persisters are phenotypic variants that constitute small subpopulations of hyper-tolerant cells that can transiently survive exposure to lethal doses of antibiotics. Recent studies have shown that persister levels can be influenced by post-antibiotic events. Specifically, it was found that inhibition of transcription or translation, or starvation of cells after fluoroquinolone (FQ) treatment significantly increased persister levels. While it had been observed that chloramphenicol (CM) following FQ treatment could increase persistence of ΔrecA, an earlier study found that starvation post FQ treatment did not increase persistence of ΔrecA. Notably, CM-assisted recovery of FQ persisters was eliminated when ΔuvrD was combined with ΔrecA. With these phenomena as motivation, we sought to understand differences and similarities between post-FQ starvation- and CM-assisted recovery of persistence. Our preliminary results reveal that starvation-assisted recovery was possible in ΔrecA if starvation was extended to longer periods of time. Further, we demonstrate that exposure to CM or starvation during recovery increase persistence through mechanisms that involve both homologous recombination and transcription-coupled repair machinery.

11. Role of dynamin-like proteins in extracellular vesicles secretion by *Mycobacterium tuberculosis* (Mtb) .

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Mycobacterium tuberculosis (Mtb) secretes extracellular vesicles (EVs) packaged with a variety of biomolecules such as proteins, lipids and nucleic acids. Mycobacterial EVs production is stimulated in response to iron limitation. Recently, there have been reports suggesting a role of EVs in Mtb pathogenesis. However, the mechanisms and key players involved in mycobacterial EV production remain undefined. In this study, we have used molecular genetics to identify Mtb proteins necessary for EV release under iron limiting conditions and antibiotic exposure. Our work uncovered the critical role of the isoniazid-induced, dynamin-like proteins, IniA and IniC, in Mtb EV biogenesis. We have also characterized Mtb iniA mutant and showed that EV secretion allows intracellular Mtb export bacterial components into the host environment and communicate with uninfected host cells potentially modulating cellular immune response. These findings increase understanding of the biogenesis and functions of mycobacterial EVs and open new avenues to target vesicle production in vivo.

12. Factors driving genome evolution of *Anisogramma anomala*, the Eastern Filbert Blight fungus, reveal lifestyle and pathogen biology

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Eastern Filbert Blight (EFB) is a devastating disease of European hazelnut (*Corylus avellana*), limiting commercial production of hazelnut in the United States. *Anisogramma anomala*, the causal agent of EFB, is a homothallic ascomycete in the Diaporthales. It has remained poorly understood due to experimental constraints with growing the pathogen in a laboratory setting. Here we report the annotated draft genome of *A. anomala* and investigate factors that are major drivers of genome evolution. The *A. anomala* genome is 350 Mbp, over 7x larger than genomes of related ascomycetes. However, *A. anomala* has 10% fewer protein coding genes compared to related pathogens. This massive genome expansion is driven by proliferation of repeat elements, for the most part identifiable transposable elements, that constitute approximately 88% of the genome. In addition to transposon-mediated genome evolution, anti-transposon genome defense mechanisms were investigated for their effect on the *A. anomala* genome. Repeat-induced point (RIP) hypermutation recognizes locally duplicated sequences and induces C --> T point mutations through a methyl-transferase. The *A. anomala* genome encodes homologues of the genes involved in RIP, including rid and dim1 in *Neurospora crassa*. Dinucleotide frequencies reveal evidence of RIP in approximately 50% of sequences distributed throughout the genome. Together, transposon and RIP activity have contributed to shaping the genomic landscape of *A. anomala* resulting in a "two-speed" genome. This is displayed as alternating blocks of non-repetitive, gene-rich regions and highly repetitive gene-poor regions with high instance of RIP activity. The non-repetitive, GC-equilibrated regions account for 7% of the genome and encode approximately 90% of protein coding genes, including highly conserved housekeeping genes involved in fungal growth and metabolism. The other 93% of the genome comprises the repetitive, highly adaptable regions that encode genes predicted to be involved in virulence and host-pathogen interactions. These AT-rich genomic regions account for less than 10% of protein coding genes, but 30% of predicted effector genes. The genomic compartmentalization of these genes supports the hypothesis of an evolutionary arms race involving gene-for-gene interactions between *A. anomala* and its *Corylus* tree host. Ultimately, the drivers of genome evolution discussed here provide insight to the lifestyle of *A. anomala* as an obligately biotrophic pathogen.

13. Phosphatidate phosphatase Pah1 contains an inhibitory domain that regulates its function in yeast lipid synthesis.

Geordan J. Stukey, Gil-Soo Han, George M. Carman

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The *Saccharomyces cerevisiae* phosphatidate (PA) phosphatase enzyme, Pah1, catalyzes the dephosphorylation of PA to produce diacylglycerol, which is the precursor for the energy storage molecule triacylglycerol as well as the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine. Pah1 function is predominantly regulated by its subcellular localization through the posttranslational modification of phosphorylation and dephosphorylation. Phosphorylated Pah1 is stable in the cytosol and recruited to and dephosphorylated by the Nem1-Spo7 phosphatase complex in the nuclear/ER membrane. The dephosphorylated Pah1 associates with the membrane, recognizes the substrate PA, and catalyzes its dephosphorylation. During transition from the exponential to the stationary phase of cell growth, Pah1 translocation is increased for increased function. Using bioinformatics, we identified a novel domain (named R-domain) of Pah1 within the intrinsically disordered region between the two conserved domains. In functional analyses, the expression of Pah1 deficient in R-domain (Pah1-DR) rescued the pah1D mutant but did not require the Nem1-Spo7 activity. Mass spectrometry analysis showed that the phosphorylation state of Pah1-DR was significantly different from the WT enzyme. We examined the abundance and localization of Pah1-DR to determine the effect of the R-domain on Pah1 stability and its translocation, and constructed substitution mutations in the R-domain to identify key residues in the regulation of Pah1 phosphorylation, translocation, and function in lipid synthesis.

14. Identification and characterization of low magnesium stress-induced small proteins in *E. coli*

Sangeevan Vellappan[1,2,3], John S. Favate[2,3], Pranavi Jagadeesan[3], Debbie Cerda[2], Premal Shah[2,3], and Srujana S. Yadavalli[1,2]

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Small proteins are defined as being less than 50 amino acids (aa) in length and encoded by small open reading frames (sORFs). Advances in bioinformatics and gene expression studies have enabled the discovery of hundreds to thousands of previously unannotated small proteins in bacteria (<50 aa, archaea, and eukaryotes, including humans (<100 aa). In *Escherichia coli*, more than 150 small proteins have been documented. Despite significant progress in identifying small proteins, a major gap in our knowledge is the lack of understanding of their functions and physiological significance. To date, only a small fraction of these proteins has been studied in detail, and they have been shown to play critical roles in cell division, signal transduction, regulation of transporters, drug efflux, and stress responses. In this study, we systematically identify several small proteins induced by low magnesium stress in E. coli using a specialized ribosome-profiling method (Ribo-RET). We find a subset of 17 small proteins out of the >150 reported in *E. coli* to be upregulated under this condition. 14 of the 17 proteins are not associated with this stress condition prior to this study, and most of them are of unknown function. Using RNA-Seq, transcriptional reporter assays, and genetic tools, we investigate the transcriptional regulation of these stress-induced small proteins. Using epitope tagging, microscopy, and biochemical analysis, we also examine their localization, overexpression, and loss-of-function phenotypes. Together, this work identifies stress-specific small proteins and systematically characterizes them, providing insights into their regulation, localization, and phenotypic effects of overexpression or loss of these small proteins.

15. Evaluation of structure-activity function of a lasso peptide using high-throughput screening.

Alina Thokkadam[1], Truc Do[1], A. James Link [1,2,3]

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Lasso peptides are natural products that belong to the class of ribosomally synthesized and post-translationally modified peptides (RiPPs). A subset of lasso peptides has targeted antimicrobial activity against pathogenic bacteria. Our group recently discovered and characterized the lasso peptide ubonodin, which has antimicrobial activity against several *Burkholderia* strains. These bacteria, especially those from the *Burkholderia cepacia* complex (Bcc), can cause severe lung infections, primarily targeting cystic fibrosis patients. We were interested in cataloging the effects of all possible amino acid substitutions on ubonodin activity as well as identifying variants with enhanced antimicrobial activity. We constructed a library of single mutants and double mutants of ubonodin and developed a next-generation sequencing screening methodology to determine the effect of ubonodin substitutions on the variants' activity against their target, RNA polymerase. Data from the screen demonstrated that ubonodin tolerates a wide array of substitutions in its loop region. Additionally, the screen revealed an ubonodin variant with improved activity compared to wild-type ubonodin. Overall, the screen of ubonodin variants provides an unprecedented look at the structure-activity relationship of a lasso peptide.

17. The fast and the furious: evolution in ssDNA viruses

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phiX174, the bacteriophage, and *E. coli* are heavily studied model systems on their own and together as a host-virus system. Despite this research, phiX174 still remains an enigma regarding its evolutionary dynamics being comparable to that of ssRNA viruses.

18. Oxidative phosphorylation potentiates antimicrobial sensitivity in *Staphylococcus aureus*.

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Staphylococcus aureus is a major human pathogen that leads to significant morbidity and mortality, causing a wide variety of infections such as pneumonia, infective endocarditis, sepsis, and soft tissue infection. An effective vaccine has yet to be developed. In prior work, we conducted a Tn-seq screen in the murine airway and identified a preponderance of mutants in metabolism. In other bacteria, metabolism via cellular respiration has been connected to antibiotic resistance, such as persister strains that develop resistance due to reduced metabolic output. To identify genes in metabolism that influence antibiotic resistance, we screened 229 mutants in various metabolic genes and identified strains with mutations in oxidative phosphorylation that exhibited enhanced resistance to aminoglycosides and bactrim. No changes in vancomycin or rifampin were observed. We identified that the oxidative phosphorylation mutants were resistant due to decreased antibiotic uptake, as shown by flow cytometry-based detection using fluorescently conjugated antibiotics. Inactivation of genes in different complexes involved in oxidative phosphorylation led to varying changes in ATP output, oxygen consumption and proton motive force, indicating that resistance was not purely driven by respiration. To probe these changes further we conducted RNA-seq and metabolomics on select strains in the presence and absence of aminoglycosides. Principal component analysis of the metabolomic and RNA-seq data showed that the resistant mutants (sdhA encoding succinate dehydrogenase and qoxC encoding cytochrome quinol oxidase) were relatively unchanged by the treatment compared to WT. Tobramycin treatment in the WT strain resulted in upregulation of metabolism-related gene sets such as C5-Branched dibasic acid, TCA cycle, galactose, pyruvate, fructose, and mannose metabolism. However, the expression of ABC transporters, two-component systems, and histidine metabolism were decreased by the tobramycin. Compared to the WT strain, sdhA and qoxC had increased levels of: ABC transporters, thiamine, pyrimidine, amino sugar, and nucleotide sugar metabolisms that may contribute to their resistance. We have identified that oxidative phosphorylation drives the ability of MRSA to take-up certain bactericidal antibiotics that could be used to inform future treatment options.

19. Unique enzymatic aspartimidylation of omega-ester-containing peptides in *Actinomycetota*.

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Asparagine or aspartate residues in protein can spontaneously cyclize over an extended period, leading to the subsequent formation of isoaspartate (isoAsp). This chemical transformation, known as aspartimidylation, inserts a methylene group into the peptide backbone, often disrupting the structure and bioactivity of the protein. To revert this isoAsp formation, a wide range of organisms – from bacteria to humans – employ a repair enzyme known as protein L-isoaspartyl O-methyltransferase (PIMT). This enzyme catalyzes the rearrangement of L-isoAsp to L-Asp. Recently, we discovered a novel class of similar methyltransferases in the genomes of actinobacteria that catalyze the opposite reaction mediated by PIMTs, aspartimidylating L-Asp leading to the L-isoAsp formation. Here, we describe the enzymatic L-Asp aspartimidylation of graspetides, which are peptide natural products characterized by diverse patterns of side chain-side chain omega-ester and omega-amide linkages. Through high-throughput genome mining, we identified 1,083 graspetide biosynthetic gene clusters encoding a PIMT-like methyltransferase gene, all found within the genomes of the *Actinomycetota* phylum. Based on sequence alignment and pattern recognition, these putative graspetides with aspartimide exhibit structures remarkably different from non-aspartimidylated graspetides found across *Cyanobacteria*, *Pseudomonadota*, and *Bacteroidota*. We elucidated the structures of aspartimidylated graspetides and their intermediates by 2D NMR spectroscopy, tandem mass spectrometry, and ester-specific hydrazine-labeling experiments, revealing their complete biosynthetic pathways. Further studies with graspetide mutants demonstrated an exceptional degree of substrate specificity exhibited by the graspetide aspartyl O-methyltransferases, in stark contrast to the generalist PIMTs that can modify a wide range of peptide substrates. Our study supports the notion that actinobacteria have repurposed the aspartimidylation chemistry for a strategic, intentional purpose.

20. Metabolic adaptation of *Staphylococcus aureus* during cystic fibrosis.

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Lung infections with *Staphylococcus aureus* are common among people with cystic fibrosis (CF), and many of these patients develop chronic infections. Previous work from our group has demonstrated a critical role for *S. aureus* metabolism in acute lung infection. However, while mutations in metabolic genes permit survival in acute infection, they are dispensable in chronic lung infection. To determine if *S. aureus* isolates from patients with CF have adapted metabolically to the CF lung, we undertook a large-scale phenotyping study of 83 sputum and nasal swab isolates from 40 patients with CF aged 0-23. Validation of isolates from sputum and swab samples was evident, as sputum samples were associated with decreased FEV% and FEV(L) in patients ($p<0.05$). We observed that sputum isolates were more likely to be resistant to gentamicin (median CFUs of 68575 vs. 333; $p<0.05$) and grew better in synthetic cystic fibrosis media (SCFM2 (maximum OD600nm of 0.80 vs. 0.57, $p<0.0001$). There were no differences between sputum and swab isolates in assays examining: metabolic capacity, biofilm activity, or hemolytic activity, nor did we observe any associations between metabolic capacity, ability to grow in rich or SCFM2 media, hemolytic activity, biofilm activity, or gentamicin sensitivity amongst all the isolates. We have sequenced all of the isolates, and preliminary data show that sputum isolates are more likely to be from sequence type 5 ($p<0.05$) and clonal complex CC5 ($p<0.05$). We are currently undertaking further antibiotic resistance and stress phenotyping of strains. We will then interrogate our sequencing data to determine if there are allelic variants that correlate with our phenotypic data. At the conclusion of this study, we aim to have a better understanding of the adaptations that occur in S. aureus during chronic infections of the CF airway.

21. Queuosine biosynthetic enzyme (QueE) moonlights as a cell division regulator.

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QueE, a key biosynthetic enzyme in the queuosine (Q) tRNA modification pathway, also mediates antimicrobial peptide stress response in *Escherichia coli.* In response to sub-lethal concentrations of cationic antimicrobial peptides, *E. coli* cells upregulate the expression of QueE, resulting in filamentous growth. This phenotype is dependent on the two-component signaling system PhoP-PhoQ, which can sense and respond to the presence of specific cationic antimicrobial peptides and other signals including low magnesium, low pH, and osmotic upshift. In our current study, we observe that the level of QueE expression controls the cell division frequency. We investigate whether the role of QueE as a regulator of cell division is functionally distinct from its role in Q-biosynthesis and translation. Using alanine scanning mutagenesis, we analyze the effects of single Ala mutants of select residues in QueE on Q-biosynthesis and filamentation. We identify specific residues in QueE that affect either its function in Q formation or cell division only, indicating that QueE is a moonlighting protein. Based on structural alignments of *E. coli* QueE and its orthologs, we identify a 22 amino acid region spanning E45-W67 that is unique to *E. coli* QueE but absent in the orthologs of *Bacillus subtilis* and *Pseudomonas aeruginosa*. Interestingly, our data show that this region (E45-W67) is dispensable for QueE's catalytic function in Q-biosynthesis but required for QueE's ability to modulate septation. Furthermore, we find that the dual roles of QueE are conserved among *E. coli* and closely related gamma proteobacteria.

22. Discovery, characterization, and transport pathway of cloacaenodin, an antimicrobial lasso peptide with activity against *Enterobacter.*

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We used genome mining to discover a new lasso peptide gene cluster encoded within species of *Enterobacter*, related to the pathogenic *Enterobacter cloacae* complex. *Enterobacter* is the second E in the ESKAPE pathogens, which are notorious for their resistance to last-resort antibiotics and urgently require new treatment options. Using genetic refactoring strategies, we produced the lasso peptide, named cloacaenodin, using heterologous expression in *E. coli.* We employed mass spectrometric and NMR analysis to show that cloacaenodin has a threaded lasso fold. This shape endows threaded cloacaenodin with proteolytic resistance, while unthreaded cloacaenodin is readily proteolyzed by common proteases. We tested cloacaenodin against a panel of *Enterobacter* strains and found that the peptide has low-micromolar antimicrobial activity against select strains, including antibiotic-resistant strains that had been isolated from clinical settings. We also used a genetic approach to decipher the transport mechanism that cloacaenodin uses to access the cytoplasm of susceptible bacteria.