CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

CIHMID

SUMMER SYMPOSIUM 2020

Friday, August 7, 2020

9:00 am – 4:00 pm

ZOOM Webinar

Register HERE

(https://cornell.zoom.us/webinar/register/WN_u-GtOdpMS_qevtoFvqF0OQ)

Cornell University

Schedule of Events

- **Each talk has a brief q&a period built into the presenter's slot.**
- **A 30- to 60-second transition slide will appear between presenters.**
- **Talks will automatically begin on the minute mark indicated.**

9:00 **CIHMID Summer Symposium goes LIVE**

9:00 – 9:06 Opening Remarks (Brian Lazzaro, CIHMID Director)

9:06 – 9:25 Morgan Eisenlord (Ecology and Evolutionary Biology, CALS)
"Eelgrass wasting disease is highly transmissible through the water column in nature and infects at a low dose over a wide temperature range"

9:26 – 9:45 Olivia Graham (Ecology and Evolutionary Biology, CALS)
"Evaluating the role of altered Zostera marina (eelgrass) microbiomes on pathogenic response"

9:46 – 10:05 Shannon Murphy (Microbiology, CALS)
"Elucidating the role of horizontally acquired genes in a cholera pandemic strain"

10:06 – 10:25 Chase Mayers (Plant Pathology and Plant Microbe Biology, CALS)
"An ancestral novel endosymbiotic bacterial symbiosis is associated with increased diversification and unique life history traits in the arbuscular mycorrhizal fungi family Gigasporaceae"

10:25 – 10:40 **"COFFEE BREAK"**

10:40 – 10:59 Surya Saha (Boyce Thompson Institute)
"Infrastructure for battling the Citrus Greening (HLB) disease: High quality genomes and an open access integrated systems biology portal"

11:00 – 11:19 Jon Sanders (CIHMID Postdoc)
"Towards a distributed framework for microbial ecophylogenomics"

11:20 – 11:39 Suzi Varvayanis (Cornell University Graduate School)
"Professional development and its effects on research productivity"

11:40 – 11:59 Mark Gallardo (Entomology, CALS)
"Targeting disease vector fertility: utilizing the CRISPR/Cas13a system to silence potential fertility genes in male Aedes aegypti mosquitoes"

12:00 – 12:59 "LUNCH"

1:00 – 1:19 Garrett League (Entomology, CALS)
"The impact of Mating and Sugar Feeding on Aedes aegypti Blood Feeding"

1:20 – 1:39 Maria Teresa Reinoso-Pérez (Ecology and Evolutionary Biology, CALS)
"Ectoparasitism during an avian disease outbreak: An experiment with Mycoplasma-infected house finches and ticks"

1:40 – 1:59 Sabrina McNew (EEB, Lab of Ornithology)
"Host transcriptional responses to pox virus infection"

2:00 – 2:19 Andrew Brodrick (Baker Institute, CVM)
"Viral factory-like structures formed by expression of reovirus nonstructural protein μNS-GFP show properties consistent with liquid-liquid phase transitions"

2:19 – 2:30 "COFFEE BREAK"

2:30 – 2:49 Vanika Gupta (Entomology, CALS)
"Heterogeneity in the fat body tissue revealed using single-cell RNA sequencing"

2:50 – 3:09 Gabrielle Le-Bûry (CIHMID Postdoc)
"Transcriptional profiling of HIV-1 infected human alveolar macrophages by single-cell RNA sequencing"

3:10 – 3:29 Kayley M. Wilburn (Microbiology and Immunology, CVM)
"Inducing cAMP production in Mycobacterium tuberculosis is sufficient to stall cholesterol utilization and shows therapeutic potential"

3:30 – 3:49 Karla García-Martínez (Microbiology and Immunology, CVM)
"Mammary Cancer Extracellular Vesicles Drive Macrophage Coagulation and Reduce Antibacterial Immunity"

3:49 – 4:00 Closing Remarks from CIHMID Director Brian Lazzaro

TALK ABSTRACTS

Viral factory-like structures formed by expression of reovirus nonstructural protein μNS-GFP show properties consistent with liquid-liquid phase transitions

Andrew Brodrick, Antonio Saporito, Meleana Hinchman, John S. L. Parker

During mammalian orthoreovirus infection, structures called viral factories (VFs) form in the cytoplasm of host cells – so termed because the presence and agglomeration of their constituents are required for viral replication and assembly of new virions. The nonstructural μNS protein is critical for VF formation and replicative functions. Ectopic expression of μNS in cells causes structures similar to VFs form in the cytoplasm. Using live cell microscopy, we studied the dynamics of a μNS-GFP fusion protein within uninfected cells. We find that μNS-GFP forms viral factory-like structures (VFLs) that undergo fusion and fission and show viscoelastic properties consistent with biomolecular condensates or liquid-liquid phase transitions. Using fluorescence recovery after photobleaching we find that the μNS-GFP molecules within VFLs are in two forms. Less than 20% of the μNS-GFP molecules have fast diffusion kinetics consistent with a dimeric form. The remaining μNS-GFP molecules show a slow recovery with a half-life of approximately 10 seconds. Although μNS-GFP VFLs exhibited both fission and fusion events, over time there was an increase in average VFL size with concentration in the perinuclear region; this perinuclear concentration was microtubule-dependent and was driven by the net movement of vesicles through VFLs dragging them towards the perinuclear region – a phenotype inhibited by nocodazole treatment. During viral infections, the morphologies and properties of VFs change. We propose a model wherein μNS VFLs are biomolecular condensates, and progressive changes in the dynamics of VFs over the course of infection are in part driven by μNS phase transitions affecting the properties of these condensates. Early in infection liquid-liquid transitions would predominate. Later as μNS interacts with other viral proteins and assembling virions, liquid-gel transitions and gel-solid transitions may occur. In larger VFs, we hypothesize that a mixture of phase transitioned μNS condensates exist.

Eelgrass wasting disease is highly transmissible through the water column in nature and infects at a low dose over a wide temperature range

Morgan E. Eisenlord[1*], M. Victoria Agnew[2], Bryanda Wippel[3], Alex Vompe[4], Miranda Winningham[1], Carolyn Friedman[3], C. Drew Harvell[1], Colleen A. Burge[2]

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Eelgrass wasting disease (EGWD), caused by the opportunistic marine pathogen *Labyrinthula zosterae* (Lz), has the potential to devastate important seagrass habitats worldwide, yet little is known about mechanisms of transmission. Outbreaks of EGWD have caused catastrophic losses of *Zostera marina* (eelgrass) globally. In this study, we examine Lz transmission and infectivity through a series of laboratory and field experiments. The infectious dose of Lz through waterborne exposure was assessed in a controlled laboratory experiment. We measured infectivity of Lz on eelgrass shoots exposed to six cell concentrations at two temperatures (7.5C and 15C). Lz infected at a concentration of as little as 10 cells/ml in 72 hours. Time to infection was delayed in the 7.5C treatment suggesting transmission is mediated by water temperature. To test waterborne transmission in the field, we out-planted hydroponic sentinel eelgrass shoots inside and outside of eelgrass beds in the San Juan Islands, WA for two weeks. Sentinel eelgrass shoots visually free of EGWD were placed inside the bed in contact with wild plants, 100m outside the bed, and 300m further from the edge of the bed. EGWD was in equal prevalence in all three treatments, indicating clear evidence for waterborne transmission rather than transmission due to propagules from near-by eelgrass beds. Control shoots maintained in the lab during the same period had significantly lower EGWD prevalence. These results support our hypothesis that Lz is highly virulent and can readily transmit through the environment without direct contact with infected plants. The complex transmission dynamics of this disease in the context of changing ocean conditions has implications for eelgrass protection and restoration along the Pacific Coast.

Targeting disease vector fertility: utilizing the CRISPR/Cas13a system to silence potential fertility genes in male Aedes aegypti mosquitoes

Mark Gallardo, I. Alexandra Amaro, Laura Harrington

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Aedes aegypti mosquitoes are a major disease vector for several medically important pathogens including yellow fever, dengue, and Zika viruses, which cause significant global morbidity and mortality. With limited vaccines and antiviral therapies, vector control remains the focus of most Ae. aegypti-borne disease control efforts. Given the high frequency of insecticide resistance and the difficulty of controlling Aedes spp. with conventional strategies, understanding this vector's reproductive and mating biology, with a focus on developing new control targets, is imperative. The CRISPR/Cas13a system could be a useful functional tool to explore important reproduction genes. In the CRISPR/Cas13a system, a guide RNA (sgRNA) binds to the Cas13a enzyme and then guides it in targeting a gene of interest's transcript (mRNA) for cleavage. When compared to Cas9-mediated gene knockout, Cas13a is a faster and less expensive methodology for functional gene screening. Given the intermittent functionality of RNAi in Ae. aegypti, Cas13a represents an attractive alternative gene knockdown modality. A gene of interest that may reduce fertility in Ae. aegypti males is seminase (Ser 2), a trypsin-like serine protease found in the seminal fluid that is transferred to female mosquitoes during mating. In D. melanogaster this enzyme participates in an early regulatory stage of the post-mating process, initiating a protease cascade signaling pathway by hydrolyzing accessory gland proteins (LaFlamme et al., 2013). More recently, complete knockout of this gene in Bombyx mori and Plutella xylostella led to male sterility (Xu et al., 2019). Although this gene's role in male Ae. aegypti fertility remains unknown, we have recently identified potential homologs to this enzyme based on work in Ae. aegypti by Degner et al. (2019). Our ongoing experiments involve intrathoracic injection of the Cas 13a plasmid, demonstrating its expression, and targeting potential Ser 2 homologs by co-injecting designer CRISPR sgRNA. If effective transcript silencing is demonstrated, functional assays will be performed by mating gene-silenced males to wild type females and then monitoring female fecundity and fertility.

Mammary Cancer Extracellular Vesicles Drive Macrophage Coagulation and Reduce Antibacterial Immunity

Karla García-Martínez, Jingyi Chen, Tracy Stokol BVSc, Ph.D., DACVP, and Cynthia Leifer Ph.D.

Within the breast cancer tumor microenvironment (TME) there is battle between tumor cells, which promote their own growth and dampen antitumor immunity, and immune cells, which have the capacity to limit or eliminate tumor cells but are often suppressed. The most abundant immune cells found in the TME are macrophages, and multiple tumor cell-derived signals modulate macrophage function within the TME. Extracellular vesicles (EVs) are secreted by tumor cells and carry numerous proteins and molecules that control cell functions in the TME and at distant sites of potential metastasis. Previous studies showed that cancer EVs skew macrophages to an anti-inflammatory phenotype, and anti-inflammatory macrophages induce coagulation through the upregulation of procoagulant proteins. Since we previously showed that cancer EVs are themselves pro-coagulant, we used a model system of syngeneic mouse mammary breast cancer cells (E0771) with mouse bone marrow-derived macrophages *in vitro* to investigate whether mammary cancer EVs upregulate macrophage procoagulant activity and determine the effect of EV exposure on macrophage immune responses. We found that exposure to cancer EVs induces macrophage clotting and dampens intraphagosomal killing of bacteria. Mechanistically, this likely involves activation of the non-canonical inflammasome and disruption of phagosomal membranes. This study is significant because poor outcome for breast cancer patients is associated with hypercoagulation, increased incidence of thrombotic events, and increased susceptibility to surgical site bacterial infections after treatment surgery and prior to initiation of immunosuppressive chemotherapy. Our findings provide possible pathways to reduce cancer induced hemostasis and promote antimicrobial immunity.

Evaluating the role of altered Zostera marina (eelgrass) microbiomes on pathogenic response

Olivia J. Graham, Emily Adamczyk, Phoebe Dawkins, Emily Chei, Arjun Lev Pillai Hausner, Sukanya Dayal, Samantha Burke**,** Taylor Hughes, Chloe Mikles, Anna Poslednik, Omisha Manglani, Kaite Cisz, Jack Elstner, Miles McDonald, Audrey Vinton, Laura Parfrey, Drew Harvell

Eelgrass (*Zostera marina*) is a temperate seagrass species that provides valuable habitats and ecosystem services worldwide. Among one of the modern threats to eelgrass is seagrass wasting disease, caused by the marine slime-mold like protist *Labyrinthula zosterae*. Infection can cause necrotic lesions that spread across eelgrass tissue and has the potential for devastating die-offs with significant ecological and economic impacts. While there is burgeoning research on eelgrass microbiomes—the bacterial communities living on the surface of tissue in biofilms—there remains a knowledge gap on the defensive properties of these bacteria against *L. zosterae*. We examined the role of eelgrass biofilms in minimizing the prevalence and severity of seagrass wasting disease. Contrary to our predictions, we found eelgrass with intact microbiomes had higher levels of disease compared to eelgrass with manipulated microbiomes. These findings broaden our understanding of host-microbiome-pathogen interactions within the eelgrass wasting disease system.

Heterogeneity in the fat body tissue revealed using single-cell RNA sequencing

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Fat body tissue in *Drosophila* dynamically controls systemic immune responses, metabolism and detoxification, and egg provisioning. The constraints imposed by using the same tissue for multiple purposes can result in sub-optimal performance of each process, and it is largely unknown how tissues balance their roles. We hypothesized that the fat body achieves its multiple functions through division of labor resulting in cellular subpopulations performing specific functions. We used single-cell RNA sequencing to test for heterogeneity in the fat body tissue. To understand the dynamic nature of the tissue, we used flies which were either challenged with a bacterium or unchallenged, under both reproductively active and inactive conditions. The results supported our hypothesis that the fat body tissue in fruit flies consists of transcriptionally heterogeneous cell subpopulations marked by unique gene expression signatures. Our results consistently showed that the ten most abundant cell subpopulations represented about 90% of all the cells assayed. Across treatments, we found that the two most abundant subpopulations were marked by expression of genes encoding yolk proteins, which play an important role in egg provisioning. Upon bacterial challenge, the expression of immune system genes was significantly upregulated ubiquitously across all the cells in the tissue. Nevertheless, the specific immune genes expressed differed across subpopulations. Our findings demonstrate that the polyfunctional fat body achieves its multiple roles both through specialization of cellular subpopulations and dynamic tissue-wide change in gene expression.

The Impact of Mating and Sugar Feeding on *Aedes aegypti* Blood Feeding

Garrett P. League, Ethan C. Degner, Sylvie A. Pitcher, Yassi Hafezi, Erica Tennant, Priscilla Cruz, Raksha Krishnan, Stefano Segundo Garcia Castillo, Mariana F. Wolfner, and Laura C. Harrington

Aedes aegypti is a globally distributed mosquito vector of arboviruses that cause dengue, chikungunya, yellow fever, and Zika. Female mosquitoes acquire pathogens, as well as essential nutrients to produce eggs, from vertebrate host blood meals. As they approach hosts for blood, Ae. aegypti females are often intercepted and inseminated by males. Although numerous studies have examined potential effects of mating on blood feeding, they have often yielded conflicting results. To resolve these discrepancies, we examined blood feeding by virgin and mated females and controlled for potentially confounding factors that could explain the differences between prior reports. We also examined blood feeding in virgins injected with male accessory gland (MAG) fluids, which contain seminal fluid proteins that are known to induce post-mating changes in female reproductive biology. Although we found no impact of mating or MAG on the amount of blood ingested, blood digestion rates, or feeding avidity, prior sugar feeding significantly affected each of these parameters. To test the findings of our laboratory studies in a natural field setting, we examined the mating and blood feeding status of indoor resting Ae. aegypti collected in Medellín, Colombia and found that virgin and mated females were equally likely to contain blood in their abdomens (78.65% and 79.14%, respectively). Together, these results demonstrate that mating has no appreciable effect on blood feeding in Ae. aegypti and suggest that sugar feeding, a common feature of artificial laboratory culture, modulates multiple aspects of blood feeding and likely contributed to the discrepant results of prior studies.

Transcriptional profiling of HIV-1 infected human alveolar macrophages by single-cell RNA sequencing

Gabrielle Le-Bûry
CIHMID Postdoctoral Fellow, Cornell University

Macrophages are susceptible to HIV-1-infection, exhibit resistance to virally-induced programmed cell death, and have been found to harbor HIV-1 DNA in virally-suppressed individuals. Recent analysis of the distribution of HIV-1 in the cells of the human airways in HIV-1 infected human volunteers has shown that viral transcripts are detected predominantly in the alveolar macrophage (AM) population and that the presence of viral transcripts persists in individuals who are effectively virally suppressed by ART (AntiRetroviral Therapy). Furthermore, during HIV infection, the human lung becomes hyper-susceptible to Lower Respiratory Tract Infections (LRTIs), with tuberculosis (TB) now the major cause of death of those living with HIV. It has shown that this increased susceptibility endures upon ART, despite immune reconstitution.

To better understand how HIV infection impairs human lung immunity, we use single cell RNA-sequencing (scRNA-seq) approaches to analyze the host transcriptional profiling in the macrophage populations in individuals who are ART-suppressed and those that are ART-naïve.

Heterogeneity in the fat body tissue revealed using single-cell RNA sequencing

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Arbuscular mycorrhizal fungi (AMF, Glomeromycotina) are root-associated fungi that are significantly important to the health of nearly all land plants worldwide. In contrast to other members of the Mucoromycota, for which endobacterial symbioses are sparse and restricted to specific lineages, symbiosis with cytoplasm-inhabiting endobacteria appears to be the rule and the ancestral state for all AMF lineages. The vast majority of AMF-endobacteria symbioses are with '*Candidatus* Moeniiplasma glomeromycotorum' (*Ca*Mg), a relative of animal-parasitic mycoplasmas that is putatively an ancestral AMF endosymbiont. Yet, specific benefits of *Ca*Mg to the host remain elusive. However, one family of AMF, the Gigasporaceae, universally and uniquely host a second endosymbiotic bacterium either in place of or in addition to *Ca*Mg: '*Candidatus* Glomeribacter gigasporarum' (*Ca*Gg). Unlike *Ca*Mg, *Ca*Gg is known to benefit host fungus spore germination and fitness and to alter metabolism. We confirmed that *Ca*Gg symbiosis is unique to the Gigasporaceae by surveying across the entire taxonomic diversity of AMF using diagnostic PCR primers for the endobacterium. We also found that members of the Gigasporaceae have a higher diversification rate than any other AMF family as a result of both increased speciation and decreased extinction rates. Members of the Gigasporaceae are exceptional among AMF in having dramatically different life history strategies (*K*-reproducing competitors with small numbers of large, high-investment offspring) compared to most other AMF (*r*-reproducing ruderals with large numbers of small, low-investment offspring). We hypothesize that establishing symbiosis with *Ca*Gg, which may have been primed by adaptation to symbiosis with the putatively-parasitic CaMg, gave the ancestral Gigasporaceae a competitive advantage or access to new ecological niches, which led to widespread diversification. *Ca*Gg symbiosis then either facilitated, or was facilitated by, dramatic shifts to the unique life history strategies in Gigasporaceae observed today. Therefore, symbiosis with *Ca*Gg is not only important to understanding the biology of the Gigasporaceae, but to understanding the evolution and development of the Glomeromycotina as a whole.

Host transcriptional responses to pox virus infection

Sabrina McNew
EEB, Lab of Ornithology Postdoc, Cornell University

Anthropogenic changes to the environment present novel challenges for wildlife. The Galápagos Islands are a useful natural laboratory for studying these changes because the relatively recent arrival of humans to the archipelago means that the effects of colonization can be studied almost in real time. A current introduced threat to Galápagos birds is *Avipoxvirus,* a virus that causes lesions on the feet and face of infected individuals, impeding foraging and mobility. Here, I present preliminary results from a transcriptomic study of the effects of pox virus infection on gene expression of two species of endemic finch: the medium ground finch (*Geospiza fortis*) and the vegetarian finch (*Platyspiza crassirostris*). Finches were captured in the wild on Santa Cruz Island in 2019. In the field, they were visually assessed for pox lesions and a small blood sample was taken via brachial venipuncture. We used RNA-seq to characterize expression profiles of 40 birds, including 10 infected and 10 uninfected individuals for each species. We recovered information on expression levels for ~ 16,000 genes across individuals. Differential expression analysis revealed clear differences in gene expression between species, but more limited differences between infected and uninfected individuals. No genes were significantly differentially expressed between infected and uninfected vegetarian finches; however, about 40 genes were significantly differentially expressed between infected and uninfected ground finches. Gene set enrichment analysis suggests that antiviral activity related to the production of interferon may be involved in host response to infection. Ongoing analyses are using “metatranscriptomic” approaches to identify parasite and pathogen transcripts in the dataset in order to quantify viral load. Studying host responses to infection may help understand why some populations are more vulnerable to emerging diseases than others and identify mechanisms of host defense.

Elucidating the role of horizontally acquired genes in a cholera pandemic strain

Shannon Murphy[1,2], Tobias Dörr[1,2]
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Vibrio cholerae is the causative agent of cholera, a notorious diarrheal disease that is typically transmitted via contaminated drinking water. The current pandemic agent, the El Tor biotype, has undergone several genetic changes that include horizontal acquisition of two genomic islands (VSP-1 and VSP-2). VSP-1 and -2 presence strongly correlate with pandemicity; however, VSP-2 remains a poorly understood 26-kb island. VSP-2 encoded genes are not expressed under standard laboratory conditions, suggesting that their induction requires an unknown signal from the host or environment. One signal that bacteria encounter under both host and environmental conditions is metal limitation. While studying *V. cholerae*'s zinc-starvation response *in vitro*, we noticed that a mutant constitutively expressing zinc-starvation genes (Δ*zur*) aggregates in nutrient-poor media. Using transposon mutagenesis, we found that disruptions to flagellar components and chemotaxis machinery prevented this aggregation, suggesting that this behavior is an active, motility-driven process. The screen additionally hit three genes on VSP-2 that appear to be transcriptionally regulated alongside other zinc-starvation genes. These VSP-2 hits encode an AraC-transcriptional activator and a methyl-accepting chemotaxis protein, the latter of which modulates bacterial movement in response to a chemical gradient. Our future work aims to characterize the chemical gradients sensed by these VSP-2 carried MCPs. Importantly, this work suggests a functional link between VSP-2 and zinc-starved environments and could yield insights into the role of VSP-2 in a metal-limited host or aquatic reservoir.

Ectoparasitism during an avian disease outbreak: An experiment with Mycoplasma-infected house finches and ticks.

Heylen D.J.A, M.T. Reinoso-Pérez, L. Goodman, K.V. Dhondt, A.A. Dhondt.

Hosts are typically co-parasitized by multiple species, and parasites can benefit or suffer from the presence of other parasites. It can reduce or increase the overall virulence due to competition or facilitation. Outcomes of new multi-parasite systems are seldom predictable. In 1994 the bacterium *Mycoplasma gallisepticum* (MG) jumped from poultry to songbirds causing an epidemic throughout USA. Songbirds are often parasitized by hard ticks, and can act as reservoirs for tick-borne pathogens. We tested the hypothesis that MG infection in house finches influences North America's most important tick vector *Ixodes scapularis*, by affecting the tick's feeding success, detachment behavior and survival to the next stage. Most ticks detached during the daylight hours irrespective of the bird's disease status and time since infestation. Birds incrementally invested in anti-tick resistance mechanisms over the course of the experiment; this investment was made earlier in the Mycoplasma-infected birds. At higher tick densities, the feeding success on birds with more severe conjunctivitis was lower than in the uninfected birds. Throughout the experiment we found positive density dependent effects on the tick's feeding success. More diseased hosts suffered more from the tick infestations, as shown by reduced haematocrits. Three Mycoplasma-infected birds died during the weeks following the experiment, although all birds were kept in optimal housing conditions. Mycoplasma made the bird a less accessible and valuable host for ticks, which is an example of ecological interference. Therefore, Mycoplasma has the potential to ultimately reduce transmission outcomes of tick-borne pathogens via songbird hosts.

Infrastructure for battling the Citrus Greening (HLB) disease: High quality genomes and an open access integrated systems biology portal

Mirella Flores-Gonzalez[1], Prashant S. Hosmani[1], Susan Brown[2], Lukas A. Mueller[1] and Surya Saha[1]
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Rapidly spreading invasive diseases in systems with little or no prior experimental data or resources pose a unique set of challenges for growers, scientists as well as regulators. As a part of a USDA NIFA CAPS project focused on the psyllid, Diaphorina citri, we have released improved genomics resources including high quality genome assemblies and annotation. We have also created an open access web portal for analyses around the Citrus Greening/Huanglongbing disease complex. Citrusgreening.org includes pathosystem-wide resources and bioinformatics tools for multiple Citrus spp. hosts, the Asian citrus psyllid vector (ACP, Diaphorina citri), and multiple pathogens including Candidatus Liberibacter asiaticus (CLas). To the best of our knowledge, this is the first example of a database to use the pathosystem as a holistic framework to understand an insect transmitted plant disease. Users can submit relevant data sets to enable sharing and allow the community to leverage their data within an integrated system. The system includes the metabolic pathway databases CitrusCyc and DiaphorinaCyc with organism specific pathways that can be used to mine metabolomics, transcriptomics and proteomics results to identify pathways and regulatory mechanisms involved in disease response. The Psyllid Expression Network (PEN) contains expression profiles of ACP genes from multiple life stages, tissues, conditions and hosts. The Citrus Expression Network (CEN) contains public expression data from multiple tissues and conditions for various citrus hosts. All tools connect to a central database. The portal also includes electrical penetration graph (EPG) recordings, information about citrus rootstock trials and metabolomics data in addition to traditional omics data types with a goal of combining and mining all information related to the Huanglongbing pathosystem. User-friendly manual curation tools will allow the continuous improvement of knowledge base as more experimental research is published. The portal can be accessed at https://citrusgreening.org/.

Towards a distributed framework for microbial ecophylogenomics

Jon Sanders
CIHMID Postdoctoral Fellow, Cornell University

Animals live enmeshed in a network of living microbes. The ability to identify and follow microbial taxa across environments via target gene sequencing has unveiled a remarkable array of patterns among this diversity. But despite these tremendous strides in ecological observation, the evolutionary dynamics of microbes in most of these natural systems remain unknown, in large part due to the difficulty of obtaining reliable genome-level information spanning relevant scales of geography, host diversity, and time. Here, I describe our recent efforts to obtain population-scale genome-resolved data from non-model systems in order to reconstruct the genomic evolutionary dynamics of their gut microbes. Focusing on the *Peromyscus* genus of deer mice as a case study, I show how we can combine automation, custom fabrication, and nanopore sequencing to obtain highly resolved genomic data from novel bacteria across many host species in a time- and cost-effective manner.

Professional development and its effects on research productivity

Based on: (accepted 2020 CBE Life Sciences Education) **Applying Experiential Learning to Career Development Training for Biomedical Graduate Students and Postdocs: Perspectives on Program Development and Design**. Audra Van Wart[1]*, Theresa C. O'Brien[2]*, Susi Varvayanis[3], Janet Alder[4], Jennifer Greenier[5], Rebekah L. Layton[6], C. Abigail Stayart[7], Inge Wefes[8], Ashley E. Brady[9]

[1]Fralin Biomedical Research Institute and Virginia Tech Carilion School of Medicine, Virginia Tech* Current affiliation: Brown University; [2]University of California San Francisco*; [3]Cornell University; [4]Rutgers University; [5]University of California Davis; [6]University of North Carolina, Chapel Hill; [7]University of Chicago; [8]University of Colorado Denver|Anschutz Medical Campus; [9] Vanderbilt University (*co-first authors)

PhD-trained scientists are essential contributors to the workforce in diverse employment sectors that include academia, industry, government, entrepreneurial and non-profit organizations. Hence, best practices for training the future workforce are of national concern. To complement traditional coursework and research training, many institutions, including Cornell, now offer career and professional training that enables career exploration and exposure, as well as the development of a broad set of valuable skills critical to the various career paths.

Experiential learning is an effective educational tool across many academic disciplines, including career development. Nine different institutions bridged by the NIH Broadening Experiences in Scientific Training (BEST) consortium compared their experiments in rethinking and expanding training of predoctoral graduate students and postdoctoral scholars to include experiential learning opportunities. This presentation provides an overview of the four major types of experiential learning, compares the learning objectives and evaluation strategies employed for each type, and includes key factors for shaping these activities on an institutional level.

An initial concern, since doctoral training is lengthy and requires focused attention on dissertation research, was that having students participate in additional complementary training activities might lengthen time to degree and hamper student research productivity. To address this concern, in a separate study, ten diverse institutions analyzed time to degree and publication records as measures of efficiency and productivity. Comparing doctoral students who participated to those who did not participate, the meta-analysis reveals that there were no differences in time to degree or manuscript output (as measured by first-author and total publications). We conclude that participation in these types of activities does not adversely affect the training timeline or productivity of doctoral students, given that these results replicated across ten different institutions offering diverse professional development opportunities.

Participation in experiential career and professional development opportunities can ensure preparedness for a variety of diverse and equally important careers in the workforce without lengthening the time to degree or hindering research productivity.

Inducing cAMP production in *Mycobacterium tuberculosis* is sufficient to stall cholesterol utilization and shows therapeutic potential

Kaley M. Wilburn[1], Christine R. Montague[1], Theresa L. Southard[2], Michael Petrassi[3], and Brian C. VanderVen[1]

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Tuberculosis (TB) chemotherapy involves multi-drug combination regimens that require months of administration to effectively prevent relapse and can have toxic side-effects. Adding to the challenge of controlling TB is the emergence of drug resistant isolates that threaten to render current control measures obsolete. Thus, identifying new drug candidates with novel mechanisms of action that simplify or shorten current TB treatments will address a major unmet need in TB control measures. Various studies have revealed that the causative agent of TB, *Mycobacterium tuberculosis* (Mtb), utilizes cholesterol to maintain optimal growth during infection. We previously identified a collection of compounds that inhibit growth of Mtb in its primary host cell niche—macrophages—and in media containing cholesterol. Surprisingly, inhibition by these compounds depends on the Mtb adenylyl cyclase Rv1625. From this series, we focused on a single compound (V-59) to investigate this mechanism of action further.

Our results demonstrate that V-59 chemically activates Rv1625c in Mtb, which stimulates cAMP production and blocks cholesterol utilization in this bacterium. Unexpectedly, we found that the six-helical transmembrane domain of Rv1625c is necessary for the complete degradation of cholesterol in Mtb, thereby linking the target of V-59 directly to the bacterium's cholesterol utilization pathway. Additionally, we found that inducing cAMP production independent of Rv1625c is sufficient to block cholesterol utilization in Mtb. Medicinal chemistry optimization of V-59 produced an orally available candidate with *in vivo* activity in mice chronically infected with Mtb. These studies establish that activating cAMP synthesis in Mtb is a novel mechanism of action that may serve as an alternative target for the development of new chemotherapies for TB. Broadly, chemically activating bacterial AC enzymes is an unconventional antibacterial target, and to our knowledge this study is the first effort to examine the therapeutic potential of this mechanism of action against a bacterial pathogen.

This work is supported by NIH grant RO1AI130018.