29th Annual Molecular Parasitology & Vector Biology Symposium

Center for Tropical & Emerging Global Diseases
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Program

8:30 AM	Registration and Poster Set-up
9:00 AM	Opening Remarks: Dennis Kyle, Director of CTEGD
	SESSION 1 — Msano Mandalasi and Stephen Vella
9:10 AM	**Anat Florentin**, CTEGD and Dept. of Cellular Biology, UGA A Bacterial Complex is Required for Plastid Integrity in *P. falciparum*
9:30 AM	**Amy Bergmann**, Eukaryotic Pathogens Innovation Center, Clemson University *Toxoplasma gondii* Relies on Its de novo Heme Production for Intracellular Growth and Pathogenesis
9:50 AM	**Introduction of Alumni speaker**
9:55 AM	**Matthew Collins**, Dept. of Medicine, Emory University Epitope Targets of the Human Antibody Response to Zika Virus Infection
10:20 AM	**BREAK — POSTER VIEWING**
	SESSION 2 — Ruby Harrison and Manuel Fierro
11:00 AM	**Jayesh Tandel**, Dept. of Microbiology, Virology & Parasitology and Dept. of Pathobiology, University of Pennsylvania Lifecycle Progression and Sexual Development of the Apicomplexan Parasite *Cryptosporidium parvum*
11:20 AM	**Babu Tekwani**, Dept. of Infectious Diseases, Div. of Drug Discovery, Southern Research Novel Phenotypic Models for Antimalarial and Antileishmanial Screening
11:40 AM	**Wei Wang**, Center for Tropical & Emerging Global Diseases, UGA Two High-quality Reference Genome Assemblies for *Trypanosoma cruzi* TcI and TcII Strains
12:00 PM	**Introduction of Alumni Speaker**
12:05 PM	**James Morris**, Eukaryotic Pathogens Innovation Center, Clemson University Pour Some Sugar on Me: Glucose, Development, Drug Discovery, and the African Trypanosome
12:30 PM	**LUNCH — POSTER VIEWING**
	SESSION 3 — Molly Bunkofske and Nathan Chasen
1:40 PM	**Ruby Harrison**, CTEGD and Dept. of Entomology, UGA Roles of the Gut Microbiota in Adult Mosquito Biology
2:00 PM	**Manuel Fierro**, CTEGD and Dept. of Cellular Biology, UGA An ER-resident Calcium Binding Protein is a Novel Regulator of the Egress Proteolytic Cascade in Malaria Parasites
2:20 PM	**Alona Botnar**, CTEGD and Dept. of Infectious Diseases, UGA Utilizing the Phytohormone Gibberellic Acid to Investigate Dormancy Mechanisms of *Plasmodium falciparum*
2:40 PM	**Introduction of Alumni Speaker**
2:45 PM	**Tiffany Weinkoff**, Dept. of Microbiology & Immunology, College of Medicine, University of Arkansas for Medical Sciences The Role of Myeloid Cells in Vascular Remodeling during *Leishmania major* Infection
3:10 PM	**BREAK — POSTER VIEWING**
	SESSION 4 — David Cobb and Josh Butler
3:40 PM	**Michael Mills**, Dept. of Microbiology, UGA Characterization of the *Wolbachia* Effector Protein Wbm0076 in Model Surrogate Host *Saccharomyces cerevisiae*

4:00 PM **Srinivasan Ramakrishnan**, Center for Tropical & Emerging Global Diseases, UGA
A Golgi-localized Cation/Proton Exchanger in *Trypanosoma cruzi* Mediates Protein Glycosylation and Parasite Replication within the Host Cells

4:20 PM **Introduction of Alumni Speaker**

4:25 PM **Marc-Jan Gubbels**, Dept. of Biology, Boston College
Of the *Toxoplasma gondii* Basal Complex Proteome: Cell Division, Apical Annuli and Beyond

4:50 PM Adjourn

Poster Presentations

P1 **Edward D'Antonio**, Dept. of Natural Sciences, University of South Carolina Beaufort
Inhibitor Development and Structural Characterization of Glucokinases from Trypanosomatid Parasites

P2 **Nathan Chasen**, CTEGD and Dept. of Cellular Biology, UGA
The Cytostome/Cytopharynx Complex of *Trypanosoma cruzi*: Uncovering the Mechanism and Proteome of this Unusual Endocytosis Organelle

P3 **Emma Troth**, CTEGD and Dept. of Infectious Diseases, UGA
Investigation of the Enzyme Kinetics of Potential Drug Targets for the Brain-Eating Amoeba, *Naegleria fowleri*

P4 **Robert Lanier, Jr**, Dept. of Natural Sciences, University of South Carolina Beaufort
Novel Amino Sugar Inhibitors of *Trypanosoma cruzi* Glucokinase

P5 **Garrett Conner**, Dept. of Natural Sciences, University of South Carolina Beaufort
Target-based Small Chemical Library Screen Against Trypanosomatid Glucokinases

P6 **Emily Knight**, Dept. of Genetic & Biochemistry, Clemson University
Characterization of a Peroxin 3-like Protein in *Trypanosoma brucei*

P7 **Flavia Zimbres**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Polyisoprenoid Metabolism in *Plasmodium falciparum*

P8 **Edwin Pierre Louis**, CTEGD and Dept. of Cellular Biology, UGA
Role of a Secreted Effector of *Toxoplasma gondii* in Modulating the Host Cell Cycle

P9 **Joshua Butler**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Mutations in *Plasmodium falciparum* Pro-drug Activation and Resistance Esterase Mediates Resistance to a Sub-class of Sesquiterpene Dimer Antimalarial Natural Products

P10 **Andrew Ho**, School of Biology, Georgia State University
Mitochondrial Calcium Homeostasis and Apoptosis in the Protozoan *Crithidia fasciculata*

P11 **Christian Cochrane**, EPIC and Dept. of Biological Sciences, Clemson University
Putative Lysosomal Chloride Channels in *Toxoplasma gondii*

P12 **Natalie Wilson**, CTEGD and Dept. of Infectious Diseases, UGA
New Clues about Ivermectin and Its Mode of Action

P13 **Erik Neff**, Savannah River Ecology Lab, UGA
Mother Does Not Always Know Best: Effects of Methylmercury on Mosquito Oviposition Behavior

P14 **Grant Butschek**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Aptamers as Biosensing Molecules for Malaria Drug Discovery

P15 **Ana Lisa Valenciano**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Metabolic Dependency of Chorismate in *Plasmodium falciparum*

P16 **Dylon Stephens**, CTEGD and College of Pharmaceutical Sciences, UGA
Screening for Specific Inhibitors of the *Plasmodium* Protease, ClpP

P17 **Carlos Sanz-Rodriquez**, Dept. of Cellular Biology, UGA
CBL0137, a Drug Lead for Human African Trypanosomiasis, Perturbs Proteostasis in *Trypanosoma brucei*

P18 **Kojo Mensa-Wilmot**, CTEGD and Dept. of Cellular Biology, UGA
Functional Classification of Genes Involved in Mitochondrial Genome Inheritance in the African Trypanosome

P19 **Brian Mantilla**, Center for Tropical & Emerging Global Diseases, UGA
Histidine Ammonia-lyase and Its Role on pH Homeostasis in *Trypanosoma cruzi*

P20 **Miguel Chiurillo**, Center for Tropical & Emerging Global Diseases, UGA
Trypanosoma cruzi Possesses Four Different Mitochondrial Calcium Uniporter (MCU) Ca^{2+}-Conducting Subunits Important for Host Infection and Replication

P21 **Nuria W. Negrão**, CTEGD and Dept. of Cellular Biology, UGA
Characterization of a Phospholipase C-like Protein (TbPI-PLC2) from *Trypanosoma brucei*

P22 **Erica Burkman**, Dept. of Infectious Diseases, UGA
Brugia malayi MicroRNAs and Potential Targets within the Feline Host (*Felis catus*)

P23 **Brock Thornton**, Dept. of biological Sciences, Clemson University
Regulation of Physiology and Organellar Organization within the *Toxoplasma* Endolysosomal System

P24 **Katherine FLoyd**, Dept. of Biological Sciences, Clemson University
Characterization and Inhibition of Protoporphyrinogen IX Oxidase (PPO) in *Toxoplasma gondii*

P25 **Benjamin Hoffman**, CTEGD and Dept. of Cellular Biology, UGA
Scission of Mitochondrial Nucleoid is Regulated by a Protein Kinase in the African Trypanosome

P26 **Mayara Bertolini**, Dept. de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas
MICU1 and MICU2 Play an Essential Role in Mitochondrial Ca^{2+} Uptake, Growth and Infectivity of the Human Pathogen *Trypanosoma cruzi*

P27 **Subash Godar**, EPIC and Dept. of Physics & Astronomy, Clemson University
Molecular Study of Axonemal Dynein's Role in the Unique Flagellar Bend Propagation in *Trypanosoma brucei*

P28 **Garrett Cooper**, CTEGD and Dept. of Genetics , UGA
Catch Me If You Can: Identifying Fast-evolving Genes in the Genus *Cryptosporidium*

P29 **Stephen Vella**, Center for Tropical & Emerging Global Diseases, UGA
Calcium Signaling and *Toxoplasma* Motility

P30 **Melanie Key**, Dept. of Biological Sciences, Clemson University
Characterization of an Oxygen-independent Coproporphyrinogen Dehydrogenase in *Toxoplasma gondii*

P31 **Connor O'Neill**, CTEGD and Dept. of Infectious Diseases, UGA
Assembly of a *Dirofilaria immitis* Genome for Multiple Drug-Susceptible and Resistant Strains for Comparative Genomic Analyses

P32 **Kristen Dominguez**, Southeastern Cooperative Wildlife Disease Study, UGA
Molecular Characterization of the Invasive Tick, *Haemaphysalis longicornis*, and Development of a Restriction Fragment Length Polymorphism (RFLP) Assay to Distinguish *Haemaphysalis* Species

P33 **Rachel Ham**, EPIC and Dept. of Biological Sciences, Clemson University
DNA Recombinases, Rad51 and Dmc1, of *Entamoeba*

P34 **Pooja Gupta**, SREL and Warnell School of Forestry & Natural Resources, UGA
Host and Environmental Factors Differentially Affect Parasite Community Structure and Infection Dynamics in a Montane Biodiversity Hotspot

P35 **Sujay Greenlund**, Dept. of biology, Georgia State University
WD40 is More Than Just a Rust Remover: Novel WD40 Proteins with Potential Roles in Cell Regulation

P36 **Alec Thompson**, SCWDS and Department of Population Health, UGA
Population Genomics of the Invasive Tick, *Haemaphysalis longicornis*

P37 **Miryam Andrea Hortua Triana**, Center for Tropical & Emerging Global Diseases, UGA
The Inositol Polyphosphate Pathway of *Toxoplasma gondii*

P38 **Angel Padilla**, Center for Tropical & Emerging Global Diseases, UGA
Screening for Reversal/Prevention of Dormancy and/or Amastigote to Trypomastigote Stage Conversion in *Trypanosoma cruzi*

P39 **Dennis Kyle**, Center for Tropical & Emerging Global Diseases, UGA
Worms Infecting Worms: Exploring the Remarkable Diversity of Blood Fluke Larval Stages Developing in Polychaetes from the Atlantic Coast of South Carolina

P40 **Jillian Milanes**, Dept. of Genetics & Biochemistry, Clemson University
Characterization of the *Naegleria fowleri* Glucokinase: Is Disruption of Glucose Metabolism a Viable Therapeutic Approach?

P41 **Susanne Warrenfeltz**, Center for Tropical & Emerging Global Diseases, UGA
EuPathDB.org: Free, Online Resources Bringing Omics to Every Parasitologist

P42 **Pei-Tsz Shin**, CTEGD and Dept. of Infectious Diseases UGA
Wolbachia Whole-genome Sequencing in Five Different Isolates of *Dirofilaria immitis* with Different Macrocyclic Lactone Susceptibility Status

P43 **Troy Koser**, Southeastern Cooperative Wildlife Disease Study, UGA
Comparison of Different Surveillance Methods for Modeling Tick Dispersal and Pathogen Prevalence for Selected Tick-borne Pathogens in Georgia

P44 **Brooke Martin**, Dept. of Biology, Georgia State University
Essential Mitochondrial Maintenance Proteins in the Parasite *Crithidia fasciculata*

P45 **David Cobb**, Center for Tropical & Emerging Global Diseases, UGA
Combined Genetic and Chemical Approaches Uncover the Essential Redox Network of the Human Malaria Parasite *Plasmodium falciparum*

P46 **Eléonore Charrier**, CTEGD and Dept. of Infectious Diseases, UGA
Glutamate-gated Chloride Channels, Ivermectin and Pharyngeal Pumping in *C. elegans*

P47 **Ruben Arroyo Olarte**, Center for Tropical & Emerging Global Diseases, UGA
Editing of Mucin-associated Surface Proteins (MASP) by CRISPR-Cas9 in *Trypanosoma cruzi*

P48 **Kayla Buck Garrett**, Warnell School of Forestry and Natural Resources, UGA
High Diversity of Cryptic Piroplasm Infections in Raccoons from Selected Areas of the United States and Canada

A Bacterial Complex is Required for Plastid Integrity in *P. falciparum*

A. Florentin[1], D. Stephens[1], C. Brooks[1], V. Muralidharan[1]
[1]University of Georgia, Department of Cellular Biology, Center for Tropical and Emerging Global Diseases

The deadly malaria parasite, *Plasmodium falciparum*, contains a non-photosynthetic plastid known as the apicoplast, that functions to produce essential metabolites. Little is known about its biology or regulation, but drugs that target the apicoplast are clinically effective. Previous studies have identified several prokaryotic Clp (caseinolytic protease) genes, encoded by the *Plasmodium* genome. In bacteria, the evolutionary ancestors of the apicoplast, and in plants chloroplasts these proteins form complexes that degrade proteins in a proteasome-like manner to regulate key cellular processes, but their function in the apicoplast is completely unknown. Using an array of molecular tools, we genetically targeted members of the apicoplast Clp complex and generated conditional mutants of the apicoplast-localized PfClpC chaperone, PfClpP protease, PfClpR, an inactive protease subunit and PfClpS, an adaptor protein. Using these genetic tools, we showed that these genes are essential for parasite viability through their central role in maintaining a functional apicoplast. Inactivation of Clp family members leads to loss of the entire organelle and can be rescued by addition of the essential apicoplast-derived metabolite, IPP. We further demonstrated physical and genetic interactions between the Clp proteins, revealing a unique mode of regulation of Clp complex activity. We designed a genetic reporter for Clp activity to study the complex ability to degrade substrates in vivo. Finally, we are using ClpP conditional mutants to screen for antimalarial compounds that target the clp complex. These data demonstrate the essential function of Clp family members in maintaining apicoplast integrity and their potential roles as antimalarials.

Toxoplasma gondii Relies on Its de novo Heme Production for intracellular Growth and Pathogenesis

Amy Bergmann[1], Katherine Floyd1[1], Melanie Key[1], Carly Dameron[1], Iqbal Hamza[2], Zhicheng Dou[1]
[1]Clemson University, [2]University of Maryland

Toxoplasma gondii is an obligate intracellular human protozoan pathogen. During its intracellular growth, *Toxoplasma* extensively communicates with its host to acquire substances. Heme, a low molecular-weight prosthetic compound, ubiquitously conjugates with many proteins to help conduct their activities. *Toxoplasma* parasites encode an intact heme biosynthesis pathway which comprises of eight consecutive reactions for heme production. However, it remains unclear whether *Toxoplasma* uses its heme biosynthesis pathway to produce their own heme. In this study, we confirmed the functionality of the three *Toxoplasma* orthologs of heme biosynthetic genes by complementing them individually in the corresponding yeast knockouts. We also successfully knocked out or knocked down four genes residing within this pathway by CRISPR-Cas9 genome editing techniques, and evaluated their growth and acute virulence in a murine model. Our preliminary data revealed that *Toxoplasma* fully sustains its intracellular growth and pathogenesis using its own heme production. Additionally, we discovered that the heme-deficient parasites are not able to utilize extracellular heme to restore their growth, which is consistent with an observation that the *Toxoplasma* does not encode a heme transporter in its genome. Given the availability of inhibitors targeting protoporphyrinogen oxidase that catalyzes the second last reaction within the heme biosynthesis pathway, we identified 4 inhibitors showing their efficacies around 100 μM in repression of parasite growth. Overall, interference in the parasite’s heme de novo production is a promising therapeutic strategy for controlling *Toxoplasma* infection.

Epitope Targets of the Human Antibody Response to Zika Virus Infection

Matthew H. Collins[1,2], Huy A. Tu[3,4], Ciara Gimblet-Ochieng[5], Guei-Jiun Alice Liou[5], Ramesh S. Jadi[5], Stefan W. Metz[5], Ashlie Thomas[5], Benjamin D. McElvany[4], Edgar Davidson[6], Benjamin J. Doranz[6], Yaoska Reyes[7], Natalie M. Bowman[2], Sylvia Becker-Dreps[8], Filemón Bucardo[7], Helen M. Lazear[5], Sean A. Diehl[3,4], Aravinda M. de Silva[5]
[1]Department of Medicine, Emory University [2]Department of Medicine, University of North Carolina-Chapel Hill [3]Cellular, Molecular, and Biomedical Sciences Program, University of Vermont [4]Department of Microbiology and Molecular Genetics, University of Vermont [5]Department of Microbiology and Immunology, University of North Carolina [6]Integral Molecular, Inc. [7]Department of Microbiology, National Autonomous University of Nicaragua [8]Departments of Family Medicine and Epidemiology, University of North Carolina-Chapel Hill

Zika virus (ZIKV) transmission became a global public health emergency after the recent epidemic in Latin America and beyond revealed rare but dire manifestations of infection such as severe birth defects and Guillain-Barré syndrome. The emergence of ZIKV in areas where other related flaviviruses such as dengue are endemic creates challenges in accurately diagnosing infections, conducting reliable surveillance, as well as in understanding the distinguishing aspects of the host immune response to ZIKV due to antibody (Ab) cross-reactivity. Because vaccines represent a key strategy for prevention of infectious diseases and typically rely on robust antibody responses, we sought to analyze the durable antibody responses in individuals infected by ZIKV as a first flavivirus infection. We observed complex populations of antibodies that bind to epitopes on intact virions, simpler epitopes on envelope protein monomers as well as envelope subdomains. Moreover, strong neutralizing antibody responses that minimally cross-react with dengue viruses were consistently detected. To better understand the molecular determinants of the neutralizing antibody response to ZIKV and to develop tools that could aid vaccine development, we isolated two potently neutralizing monoclonal antibodies (mAbs) from one primary ZIKV case and mapped key amino acid residues involved in mAb binding and neutralization by multiple complimentary methods including generation of neutralization escape mutants and alanine scanning mutagenesis. The mAbs recognize different epitopes centered on domain I and domain II of the viral envelope protein. Functionally, both mAbs were protective in a lethal mouse model of ZIKV infection. Ongoing work is examining the prevalence of these specific Ab responses at the population level. This work provides new knowledge and tools that may be useful as diagnostic reagents or as therapeutics and will advance vaccine development.

Lifecycle Progression and Sexual Development of the Apicomplexan Parasite C*ryptosporidium parvum*

Jayesh Tandel[1,2], Elizabeth English[2], Adam Sateriale[2], Jodi Gullicksrud[2], Daniel Beiting[2], Brittain Pinkston[3] & Boris Striepen[1,2]
[1]Microbiology, Virology & Parasitology, UPenn; [2]Dept. of Pathobiology, UPenn; [3]CTEGD, Univ. of Georgia

Cryptosporidium is the second major leading cause of diarrhea-induced mortality in children, and a major risk factor for immunocompromised individuals like HIV patients. Currently, there are no drugs or vaccines against the parasite. Lack of continuous culture system and limited understanding of the molecular biology of the lifecycle are some of the limiting factors that have prevented the development of therapeutics. *Cryptosporidium* infection in initiated by ingestion of oocysts (meiotic spores). Parasites then undergo asexual replication, sexualization, mating and oocysts biogenesis within the same host. Oocysts can further infect the same host or can be transmitted to infect a naïve host. *Cryptosporidium* lifecycle have been studied extensively in vivo and in vitro. However, a detailed in vitro & in vivo comparative analysis of the lifecycle progression is lacking to pinpoint factor(s) that prevent continuous culturing of the parasite. We have used different stage-specific reporter strains of the parasite to carry out comparative lifecycle studies. We observe robust sexualization of the parasite (~80%) in the culture. However, we do not observe post-fertilization stages and de novo oocyst biogenesis in culture. Contrastingly, post-fertilization stages represent around ~30% of the total parasite population in vivo. Our independent Cre-lox based reporter assay for sex has revealed that parasites fail to mate in culture. This suggests that sex and oocyst biogenesis is required for transmission and to maintain continuous infection in the host (contrasting to the *Plasmodium* lifecycle). We have further used sex-specific reporter strain to study stage-specific transcriptional profiles. We further aim to target sex-specific genes to block oocyst development and test its effect on transmission and infection in the animal model.

Novel Phenotypic Models for Antimalarial and Antileishmanial Screening

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Department of Infectious Diseases, Division of Drug Discovery, Southern Research, Birmingham AL USA

Malaria and leishmaniasis continue to be the major global health challenges due to significant mortality and morbidity attributable to these diseases. The choice of therapies currently available for the treatment of these infectious diseases is highly limited and several of these may eventually be lost or compromised due to drug-resistance. Continuous emergence of drug resistance underscores the need for identification of new drugs; indeed, the building and continuous augmentation of an armamentarium of multiple drugs is necessary to cope with the problem of further development of resistance. Molecular targets-based screening followed by evaluation of the compounds libraries through in vitro phenotypic models and in vivo preclinical models are the hallmarks of new drug discovery.

The whole parasite cell culture-based phenotypic models have attracted significant attention due to recent development of advanced tools for cell imaging and technologies for generation of transgenic cell lines of the pathogens. New phenotypic cell-based models have been developed for *Leishmania donovani* and *Plasmodium falciparum* and *P. berghei*. The parasite-rescue and transformation model was developed for the THP1 macrophage-internalized *L. donovani* amastigotes. The transgenic cell lines of *L. donovani* were developed with stable constitutive expression of mCherry and Citrine fluorescent reporter genes. Staining of the malaria parasites with LDS-751, a fluorescent cell-permeant nucleic acid stain, was developed for parasitemia analysis in blood samples from *P. berghei* infected mice and also for in vitro *P. falciparum* cultures. These models have been validated through extensive screening of natural and synthetic compounds libraries. The new phenotypic models offer significant advantages regarding selectivity, throughput and application for compounds' library screening with additional utility for evaluation of virulence and drug-sensitivity of clinical isolates.

Two High-quality Reference Genome Assemblies for *Trypanosoma cruzi* TcI and TcII Strains

Wei Wang[1], Rodrigo P. Baptista[1,2], Duo Peng[1,3], Yiran Li[2], Todd Mining[1], Jessica C. Kissinger[1,2,4] and Rick L. Tarleton[1,3]
[1]Center for Tropical and Emerging Global Diseases; [2]Institute of Bioinformatics; [3]Department of Cellular Biology; [4]Department of Genetics, University of Georgia, Athens, GA, USA

The current reference genome for *Trypanosoma cruzi*, the causative agent of Chagas disease, is very fragmented and incomplete due to highly repetitive DNA content (50%), the hybrid nature of reference strain CL-Brener, and the short read method used for their sequencing. To generate a higher quality reference genome, a single-molecule, long-read sequencing platform (SMRT sequencing) was used to sequence two homozygous isolates, Brazil (clone 4) from TcI and Y strain (clone 6) from TcII, in order to better resolve species-wide genome complexity. Further incorporation of Chicago and Hi-C library analysis enabled the genomes to be assembled into 406 and 264 scaffolds in Brazil and Y, respectively, the highest contiguity among all *T. cruzi* genomes to date. A manual curation was performed to predict gene models for both genomes, based on several sets of evidence, and a customized pipeline was developed to predict large multi-copy gene families, which permits the identification of full repertoires of gene structures. The largest 43 scaffolds in Brazil and 40 in Y strain were assigned as chromosomes, and the additional smaller scaffolds were mostly composed of members of large gene families (e.g. trans-sialidases, mucins, MASPs, etc), and in some cases, allelic variants (despite the fact that these are non-hybrid genome). The two genomes varied in genomic sequence, gene copy number and chromosome copy numbers, further demonstrating the diversity and plasticity of genomic architecture in *T. cruzi*. Comparative analysis between these two genomes also revealed that while the overall structure of them was similar, some chromosomes which contained mainly multi-copy gene families showed completely unique composition in the respective strains. Therefore, our analysis will focus further on the divergence of these gene families under evolutionary pressure.

Pour Some Sugar on Me: Glucose, Development, Drug Discovery, and the African Trypanosome

James C. Morris
Eukaryotic Pathogens Innovation Center, Clemson University

Glucose is critical for the infectious blood stages of the African trypanosome, *Trypanosoma brucei*, serving as both a key metabolic agent and an important signaling molecule. While lack of the hexose is toxic to the proliferative long slender life stage of the parasite, the absence of glucose initiates differentiation in the non-dividing short stumpy (SS) form. These parasites demonstrate hallmarks of development into the next lifecycle stage, the procyclic form (PF) parasite, that include resumption of growth and expression of PF-specific antigens. Both SS differentiation and the growth of the resulting PF parasites is inhibited by glucose and non-metabolizable glucose analogs, with the latter observation suggesting a potential receptor-mediated mechanism for perception of the sugar. The importance of the hexose to the parasite for both metabolic an developmental needs suggests that glucose uptake or distribution inhibitors would be potentially useful anti-parasitic compounds. To identify small molecule inhibitors of glucose acquisition, we developed parasites that endogenously express FRET-based protein glucose sensors in the cytosol or glycosomes. Using these cells, we have completed a 25,000-compound pilot screen and have identified inhibitors with useful medicinal chemistry properties that have potential as a new line of lead compounds against the parasite.

Roles of the Gut Microbiota in Adult Mosquito Biology

Ruby Harrison[1,2], Bret Boyd[2], Mark Brown[1,2], Michael Strand[1,2]
[1]CTEGD and [2]Department of Entomology, University of Georgia, Athens, GA

Adult mosquitoes harbor a gut microbiota which is acquired during the juvenile life stage from the environment. This microbiota consists of a variable community of 100-200 species, predominantly bacteria. Studies indicate the mosquito gut microbiota influences physiological processes such as blood meal digestion, immune system function, and pathogen acquisition and transmission. To facilitate investigation of mosquito-microbe interactions, we developed a novel rearing method to generate axenic (microbe-free) adult mosquitoes. We determined that axenic adults were initially identical to conventionally reared controls in biological parameters such as development time, body size, and propensity to blood-feed. However, after taking a blood meal, axenics exhibited several physiological defects including impaired nutrient assimilation, disrupted endocrine function, and reduced egg formation. In addition, preliminary pathogen challenge assays using the *Aedes aegypti*-Dengue virus system indicated axenic females are more susceptible to viral infection than controls. Overall, our results confirm the gut microbiota modulates key aspects of mosquito biology, impacting functions which follow blood-feeding.

An ER-resident Calcium Binding Protein is a Novel Regulator of the Egress Proteolytic Cascade in Malaria Parasites

Manuel A. Fierro[1,2], Beejan Asady[1], Carrie Brooks[1], Alejandra Villegas[1,2], Silvia N.J. Moreno[1,2], and Vasant Muralidharan[1,2]
[1]Center for Tropical and Emerging Global Diseases, [2]Department of Cellular Biology, University of Georgia, Athen, GA

Plasmodium is the causative agent of malaria, a disease that continues to be a large burden in susceptible regions of the world. The parasite ER is thought to play a key role in egress and invasion of daughter merozoites through organelle biogenesis, protein trafficking, and Ca^{2+} signaling. However, none of the proteins responsible for these ER-related functions during egress and invasion of apicomplexan parasites have been identified. We focused our studies on the ER-resident calcium binding protein (PfERC) as it is the only protein with identifiable Ca^{2+}-binding domains in the ER and generated conditional mutants using the CRISPR/Cas9 system. Lifecycle analysis of the PfERC mutants revealed that egress from the RBC was inhibited and invasion was blocked upon knockdown. The effects that we observed were not due to general defect in ER function as organelle biogenesis and protein trafficking was not affected upon knockdown. Furthermore, Ca^{2+} measurements using the cell-permeant Ca^{2+} indicator, Fluo-4AM suggest that the ER calcium storage is not affected by knockdown of PfERC. Instead, our data show that knockdown prevented the breakdown of the parasitophorous vacuolar membrane. This was a result of defective maturation of several proteins involved in egress as well as invasion. Specifically, PfERC was essential for the proteolytic maturation of the key egress protease, SUB1. A defect in SUB1 maturation affected processing of its substrates like MSP1, which is required for both egress and invasion. Ultimately, our results show that PfERC is essential for parasite egress by regulating the proteolytic cascade required for this event.

Utilizing the Phytohormone Gibberellic Acid to Investigate Dormancy Mechanisms of *Plasmodium falciparum*

Alona Botnar[1,2], Chungsik Kim[3], Roman Manetsch[3], Dennis E. Kyle[1,2,4]
[1]Department of Infectious Diseases, University of Georgia, Athens, GA [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA [3]College of Science and the Bouve College of Health Sciences, Northeastern University, Boston Massachusetts [4]Department of Cellular Biology, University of Georgia, Athens, GA

The current gold standard treatment for malaria is artemisinin combination therapy. However, Artemisinin (ART) is known to induce dormancy in the ring stage parasite, during which the parasite is under a growth arrest, eventually resuming growth 7-10 days after drug exposure. ART induced dormancy is a stress response that allows parasites to withstand the cytotoxic effects of the drug. The mechanism by which the parasite enters this state of dormancy and later recrudesces to continue development is currently unknown. Recently, Duvalsaint and Kyle discovered that Gibberellic Acid (GA) treated parasites recovered from dormancy (expressed as resumption of normal morphology) 48 hours earlier than non-GA-treated parasites. In this study, we conducted limited structure-activity relationship studies of GA on ART-induced dormant *Plasmodium falciparum*. GA derivatives were prepared and tested using chemical biology approaches on ART-induced dormant parasites. We observed GA derivatives that activated the dormant parasites and resulted in faster recovery from dormancy as compared to control. On the contrary, we also observed that some GA derivatives prolonged dormancy. We then employed these derivatives and click chemistry methods to localize GA within *Plasmodium falciparum*. Utilizing this approach, we were able to localize the GA derivatives to the cytoplasm. Since GA is a plant hormone, the localization of GA to the cytoplasm leads us to believe that it binds to intracellular receptors residing in the cytoplasm, which then activate cell signaling pathways that have yet to be elucidated. We next plan to further our understanding by employing pull down methods and mass spectrometry to identify the GA interacting partners. These studies will broaden our scope of understanding of the dormancy mechanism of *Plasmodium* and will be important for the development of novel drugs.

The Role of Myeloid Cells in Vascular Remodeling during *Leishmania major* Infection

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Our lab investigates the mechanistic basis for the pathogenesis of disease driven by *Leishmania*, a protozoal parasite that causes cutaneous lesions. Following infection, lesion severity is often increased by an exaggerated inflammatory response. As a result, the inflammatory response can maintain disease even after the parasite infection has been controlled. Given vascular remodeling contributes to the magnitude of many inflammatory conditions, it is hypothesized that the manipulation of factors promoting angiogenesis or lymphangiogenesis would alter lesion severity in leishmaniasis. Our findings demonstrate that murine *Leishmania major* infection leads to dramatic changes in vessel morphology, number and permeability. At the peak of infection, VEGF-A and VEGFR-2 expression are upregulated and VEGFR-2 blockade led to a reduction in lymphatic endothelial cell proliferation and simultaneously increased lesion size without altering the parasite burden. We showed VEGF-A/ VEGFR-2 signaling promotes lymphangiogenesis to restrict tissue inflammation in leishmaniasis. Given VEGF-A/ VEGFR-2 signaling contributes to lesion resolution, we are currently investigating the cellular and molecular mediators driving VEGF-A production. We found that macrophages are the predominant cell type expressing VEGF-A during *L. major* infection and that parasites can directly induce VEGF-A production by macrophages in vitro. Given *Leishmania* parasites activate HIF-1α and this transcription factor induces VEGF-A expression, we analyzed the expression of HIF-1α during infection. We showed that macrophages are the major cell type expressing HIF-1α during infection and that parasite-induced VEGF-A production is mediated by HIF activation. We are presently examining VEGF-A expression in mice deficient in HIF signaling specifically in myeloid cells. To date, we have shown that $LysM^{Cre}$ $ARNT^{f/f}$ mice express less VEGF-A than $LysM^{Cre}$ $ARNT^{f/+}$ control mice following infection, and we are exploring how decreased myeloid VEGF-A production influences vascular remodeling and lesion resolution in these animals. Altogether, these studies suggest macrophage HIF-dependent VEGF-A production contributes to lymphatic remodeling during *L. major* infection.

Characterization of the *Wolbachia* Effector Protein Wbm0076 in Model Surrogate Host *Saccharomyces cerevisiae*

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Brugia malayi, a parasitic roundworm of humans, is colonized by obligately intracellular bacteria of the *Wolbachia* genus. This symbiosis between worm and bacterium is essential for nematode reproduction and long-term survival in the human host. Therefore, identifying molecular mechanisms required by *Wolbachia* to colonize *B. malayi* will provide drug targets for clearance of the parasite in lymphatic filariasis patients. *Wolbachia* type IV secretion components are implicated in the secretion of effector proteins into the cytosol of *B. malayi* host cells. However, the characterization of such *Wolbachia* secreted proteins (wSPs) has remained elusive because neither *B. malayi* nor its *Wolbachia* can be cultured individually for molecular and biochemical studies. To navigate this, bioinformatics has been employed to identify putative wSPs, which were subsequently screened for the ability to modulate the cellular biology of a model eukaryotic cell, *Saccharomyces cerevisiae*. Several putative wSPs induced defects in yeast growth, suggesting possible functions in a eukaryotic cell. Strikingly, expression of the toxic wSP, Wbm0076, resulted in an increase of cortical actin patch formation in yeast, as measured by fluorescently-tagged actin binding proteins, Abp1p and Abp140p. Furthermore, we find that yeast strains lacking Abp1p, an Arp2/3-binding protein, are resistant to Wbm0076 toxicity. Wbm0076 displays homology to the family of Wiskott-Aldrich syndrome proteins (WASp) that recruit and activate the conserved Arp2/3 protein complex to nucleate actin monomers (G-actin) and initiate the polymerization of filamentous actin (F-actin). Indeed, further probing of putative WASp motifs suggest that Wbm0076 may function as a WASp-like protein to modulate eukaryotic actin dynamics. Investigation of Wbm0076 toxicity in yeast, particularly analysis of its protein and actin binding activities via biochemical assays, will be instrumental in characterizing and elucidating the function of Wbm0076; thus providing molecular insight into a mechanism by which *Wolbachia* persists in *B. malayi*.

A Golgi-localized Cation/Proton Exchanger in *Trypanosoma cruzi* Mediates Protein Glycosylation and Parasite Replication within the Host Cells

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Trypanosoma cruzi is a protist parasite and the causative agent of American trypanosomiasis. The parasite life cycle can be simply partitioned into an insect stage and a mammalian stage. Many key biochemical processes facilitate the transition from the insect stage to the mammalian stage. One such process is protein glycosylation. Glycosylated proteins play a key role in host-parasite interaction and facilitate adhesion, invasion and immune evasion. Recently, a Golgi-localized Mn^{2+}-Ca^{2+}/H^{+} exchanger was discovered in yeast and humans and was shown to be required for efficient protein glycosylation. An ortholog for this protein can also be found in the *T. cruzi* genome. We hypothesized that the *T. cruz*i GDT1 could be playing an important role in the host-parasite interaction by modulating the parasite glycosylation pathway. Using immunofluorescence assays and electron microscopy we showed that this protein localizes to the Golgi of the parasite. We demonstrated that the *T. cruzi* GDT1 can complement the growth defect observed in yeast GDT1 *null* mutant. To functionally characterize this protein in the parasite, we used the recently developed CRISPR/Cas9 strategy to generate a *null* mutant for GDT1 in *T. cruz*i. The loss of GDT1 does not affect the insect stage of the parasite. But, in support of our hypothesis, the growth within the mammalian cells is severely reduced. Additionally, a defect in protein glycosylation can also be observed in this mutant. Our current and future experiments are focused on assessing the extent of glycosylation defect in GDT1 mutant parasites and how this affects the parasite growth specifically within the host cells.

Of the *Toxoplasma gondii* Basal Complex Proteome: Cell Division, Apical Annuli and Beyond

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Toxoplasma gondii replicates by an internal budding mechanism producing two daughter parasites per division round. Budding is driven by cortical cytoskeleton assembly and concludes with the actions of the basal complex (BC). Although the BC is reminiscent of the contractile ring in higher eukaryotes, its composition, mechanism and controls differ substantially. To deepen our insights in this unusual cytokinesis apparatus, we dissected its proteomic composition by reciprocal proximity-dependent biotinylation experiments (BioID). This identified numerous undefined proteins, several of which with critical roles in cell division, next to hints at multiple phosphorylation-based controllers. Next, we assembled a protein-protein interaction network using interaction probability predictions, which defined several sub-complexes as well as protein hubs connecting the complexes. Furthermore, temporal resolution across the budding process revealed components uniquely associated with BC initiation, its expansion and, surprisingly, its mature phase, hinting at functions beyond cell division. Serendipitously, some of the BC proteins were also present in the enigmatic apical annuli, which comprise 5-6 donut shaped structures toward the basal end of the cytoskeleton. Assessment of the annuli resolved their architecture and provided hints toward a function in internal budding, thereby highlighting an underappreciated aspect of cell division.

Poster Presentations

P1. Inhibitor Development and Structural Characterization of Glucokinases from Trypanosomatid Parasites

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Chagas' disease and leishmaniasis are life-threatening infections caused by human pathogenic parasites such as *Trypanosoma cruzi* and *Leishmania* spp., respectively. Available drugs as treatment options have substantial limitations including intolerability, adverse side effects, and toxicity concerns. A potential drug-target in these trypanosomatid parasites is glucokinase, an enzyme at a nodal position between two critically important pathways, glycolysis and the pentose phosphate pathway. The enzyme phosphorylates D-glucose in the presence of ATP to form products G6P and ADP. The inhibition of glucokinase from a trypanosomatid parasite appears to be an important drug discovery strategy. The objectives were centered on the X-ray crystal structure determination of *T. cruzi* glucokinase (*Tc*GlcK) crystal structure complexes of competitive inhibitors, determination of the first crystal structure for a glucokinase from a species of *Leishmania* that includes *L. braziliensis* glucokinase (*Lb*GlcK), and small-molecule screening assays versus these same enzymes. The following procedures were performed: (a) the expression/purification of the glucokinases, (b) enzyme activity assays, (c) primary screening/counter assays, and (d) crystallization/X-ray crystallography. Our research group determined the 3-dimensional high-resolution X-ray crystal structures for *Tc*GlcK in complex with four competitive inhibitors, and also, *Lb*GlcK, the first reported glucokinase structure for a *Leishmania* spp. Structural comparisons for *Tc*GlcK and *Lb*GlcK will each be compared against *Homo sapiens* hexokinase IV so that further insights into structure-based drug design (SBDD) can be fully appreciated. A high-throughput screening campaign along with small libraries of leading scaffolds were tested to screen *Tc*GlcK. Lead hits from these screens were also evaluated for anti-*T. cruzi* biological activity. We will also present the results of a small inhibitor library screening run against *Lb*GlcK. Lead molecules identified from the *Tc*GlcK – inhibitor screens and the *Lb*GlcK – inhibitor screens will be the subject of future SBDD and/or structure-activity relationship studies for the optimization of efficacious antiparasitic drugs.

P2. The Cytostome/Cytopharynx Complex of *Trypanosoma cruzi*: Uncovering the Mechanism and Proteome of this Unusual Endocytosis Organelle

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Trypanosoma cruzi, the causal agent of Chagas' disease, spends the pathogenic portion of its lifecycle primarily in the cytoplasm of mammalian host cells as actively replicating amastigotes. We know surprisingly little about how *T. cruzi* interacts with its host cell in terms of the mechanistic basis of metabolite uptake from the host cytoplasm. Our group has, for the first time, identified a number of proteins associated with the cytostome/cytopharynx complex (CSP), a long tubular invagination of the parasite membrane that is the primary site of amastigote and epimastigote endocytosis and thus an important route of nutritional uptake. We have demonstrated that endocytosis of BSA, Transferrin, and Concanavalin A all occur along the CSP structure labeled by these proteins. Our group has also taken the first steps toward understanding the mechanism for material transport through the CSP, via the identification of a motor protein, found along the entire length of CSP, and showing that actin polymerization inhibitors prevent endocytosis into the CSP. The goal of this work is to thoroughly characterize the CSP and its endocytosis mechanism, with the hope of informing potential therapeutic strategies for the intracellular amastigote stage that is responsible for Chagas' disease pathology.

P3. Investigation of the Enzyme Kinetics of Potential Drug Targets for the Brain-Eating Amoeba, *Naegleria fowleri*

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Naegleria fowleri is a pathogenic free-living amoeba that is commonly found in warm, freshwater and can cause a rapidly fulminant disease known as primary amoebic meningoencephalitis (PAM). The fatality rate of PAM is >97%, thus there is an emphasis on innovative drug development. Until recently, few advances have been made in the discovery of new drugs for PAM and other diseases caused by pathogenic free-living amoebae. We aim to identify and validate novel drug targets for *N. fowleri*. We aim to develop high-throughput biochemical screening methods to identify drugs that specifically inhibit *N. fowleri* proteins. Currently, our work is focused on developing assays for *N. fowleri* glucokinase, Rho GTPase, and deoxyhypusine synthase. In order to develop sensitive biochemical assays, the enzyme kinetics of these potential drug targets must first be determined. KM and Vmax are essential enzymatic properties that must be determined prior to final assay optimization. The KM and Vmax for *N. fowleri* Rho GTPase and deoxyhypusine synthase are unknown and will be determined prior to screening. We are employing the ADP-Glo, GTPase-Glo, and NADH-Glo assays (Promega) to develop our screen. Upon optimization of these high throughput assays, we plan to screen multiple drug libraries, which include already FDA-approved compounds. Drug hits from these screens will be taken in to further development.

P4. Novel Amino Sugar Inhibitors of *Trypanosoma cruzi* Glucokinase

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Trypanosoma cruzi is a protozoan parasite that causes Chagas' disease and has affected approximately 6 – 7 million people worldwide, but primarily in Latin America. Glycolysis is an essential pathway for the parasite and our laboratory has an interest in one of the glycolytic enzymes, such as *T. cruzi* glucokinase (*Tc*GlcK). This study focused on the synthesis and evaluation of *Tc*GlcK inhibitors since the enzyme is a potential drug-target of the organism. To identify a class of monosaccharide-based inhibitors that are potent and selective with respect to the human homologue, a focus was centered on the structure-activity relationship from a previously confirmed inhibitor of *Tc*GlcK that also featured trypanocidal activity, namely, benzoyl glucosamine (BENZ-GlcN). We expanded on a small chemical library that consisted of 17 amino sugar analogues of BENZ-GlcN to potentially inhibit *Tc*GlcK. Three monosaccharide scaffolds were explored, including: D-glucosamine, D-mannosamine, and D-galactosamine. A thorough early-stage drug discovery analysis was carried out to identify potential hit-to-lead candidates. This involved screening the library against various factors: (a) *Tc*GlcK, (b) *Homo sapiens* hexokinase IV (a human homologous enzyme representative), and (c) the in vitro *T. cruzi* infective form (i.e. amastigotes and trypomastigotes) co-cultured in NIH-3T3 murine host cells. The results of the lead inhibitors will be presented that addresses the molecular aspects of *Tc*GlcK inhibition through structure-activity relationships.

P5. Target-based Small Chemical Library Screen Against Trypanosomatid Glucokinases

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Chagas' disease and leishmaniasis are two neglected tropical diseases of the world that lack effective therapeutic treatment options and cause life-threatening complications. A need exists for new and improved drug options for both diseases. Our laboratory recently identified a cluster of hits having the same core scaffold from a high-throughput screen (HTS), which inhibits *Trypanosoma cruzi* glucokinase (*Tc*GlcK) quite well (e.g. two inhibitors had IC50 values in the low micromolar range). *Tc*GlcK is a potential drug target of the *T. cruzi* protozoan parasite because it is an enzyme found at a nodal point between two critically important metabolic pathways, glycolysis and the pentose phosphate pathway. Compounds of the identified cluster belonged to the 3-nitro-2-phenyl-2H-chromene scaffold, and in this study, a small chemical library of analogues were purchased from commercially available suppliers that were subsequently tested against two trypanosomatid glucokinases, *Tc*GlcK and *Leishmania braziliensis* glucokinase (*Lb*GlcK) for the purpose of searching for improved inhibitors. *Tc*GlcK and *Lb*GlcK share a 44% protein sequence identity, and with enough differences between the two glucokinases, inhibitors of the chemical library are likely to inhibit at different magnitudes. The purchased compounds all had one-point changes from one of the hit-to-lead candidates of our *Tc*GlcK HTS campaign and the analysis will aid in the understanding of which regions of the core scaffold are important for inhibition. Most of the compounds examined in the primary screen of *Tc*GlcK were determined to be hits and the primary screening results of compounds against *Lb*GlcK will be reported in due course.

P6. Characterization of a Peroxin 3-Like Protein in *Trypanosoma brucei*

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Kinetoplastids have specialized peroxisomes called glycosomes that are essential. Despite their importance to parasite survival, we know little about the processes that regulate these organelles. In higher eukaryotes, peroxisomes proliferate via fission of existing organelles or de novo biogenesis from the endoplasmic reticulum; processes that are regulated by proteins called Peroxins (Pexs). Pex3 is the cornerstone of peroxisome biogenesis and has been identified in all peroxisome bearing organisms except kinetoplastids. Pex3 functions through interactions with another protein, Pex19. We have identified an open reading frame encoding a Pex3-like protein (P3L; Tb927.9.11350) in the kinetoplastid genome. This gene encodes a protein containing conserved 17 amino acid sequence (SNKLEIWEDLKIISFTR) necessary for Pex3-Pex19 interaction in Arabidopsis, humans, and Saccharomyces. Our objective is to determine if TbP3L is a Pex3 homolog. The putative Pex19 binding domain is conserved in all kinetoplastids. We expressed hemagglutinin antigen-tagged P3L (HA-P3L) in parasites. In IFA, HA-TbP3L staining overlapped with the glycosome marker protein aldolase. Several glycosome proteins were identified in organelles isolated via affinity purification using anti-HA magnetic beads. Currently, we are performing density centrifugation to determine if HA-TbP3L sediments with glycosome proteins, as well as targeted co-immunoprecipitation using HA-TbP3L as bait. These data support the hypothesis that HA-P3L is a kinetoplastid Pex3 homolog. Pex3 is essential for multiple processes of peroxisome development in higher eukaryotes and our ability to identify a homolog in kinetoplastids has prevented us from studying these processes in kinetoplastids. Validation of P3L as a Pex3 homolog would open multiple avenues for future investigations into the pathways that coordinate glycosome biogenesis.

P7. Polyisoprenoid Metabolism in *Plasmodium falciparum*

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Human malaria is a mosquito-borne infectious disease associated with a high risk of mortality. Most cases of life-threatening malaria are attributable to infection with *Plasmodium falciparum* which has a complex life cycle that involves the human host and its vector, the Anopheles mosquito. The rapid development of resistance to all current treatments highlights the urgent need for new therapies with novel mechanisms of action. The isoprenoid biosynthetic pathway is a promising source of malaria-specific drug targets, because it occurs through the methylerythritol phosphate (MEP) pathway in the apicoplast of malaria parasites, an organelle that is absent in humans. Moreover, isoprenoid products differ from those in the human host and are involved in a wide variety of vital biological functions. Nonetheless, our knowledge of polyisoprenoid function is very limited and many aspects of its biosynthesis, distribution and interaction with other cellular structures remain unexplored. In the present work, we aimed to study polyisoprenoid metabolism with a focus on dolichol biosynthesis. Dolichols are products of a reduction of the α-isoprene unit present in polyprenols by a polyprenol reductase and they are involved in protein N-glycosylation, protein O-mannosylation and GPI-anchor biosynthesis. Here, the putative polyprenol reductase gene (PfPPR) found in the Plasmodium genomics database with gene ID PF3D7_1455900 (PlasmoDB) was able to restore dolichol synthesis and protein glycosylation in the dfg10 mutant yeast strain. We also characterized the distribution of polyprenol and dolichol species present in the asexual and sexual stages of *P. falciparum*. Moreover, we knocked down *PfPPR* using a CRISPR-cas9 approach and metabolomics analysis further confirmed its enzymatic function.

P8. Role of a Secreted Effector of *Toxoplasma gondii* in Modulating the Host Cell Cycle

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As one of the most common parasitic zoonoses, the obligate intracellular protozoan parasite, *Toxoplasma gondii*, infects a third of the world population. Remarkably, this apicomplexan is also known for its capability to infect any nucleated cell of warmed blooded animals resulting in a life-long chronic infection. Subsequent to infection, *Toxoplasma* releases molecular effector proteins via several secretory organelles (micronemes, rhoptries, dense granules) that drive the host cell to undergo a rapid transcriptional reprogramming of key pathways relating to metabolism, the immune response and cell cycle processes. So far, the dense granule vesicles have been shown to harbor a myriad of host modulating nuclear targeted parasite effectors. We have identified a dense granule protein that translocates across the parasitophorous vacuole (PV) membrane, traffics to the host nucleus and is responsible for driving the infected host cell into the S¬phase of the cell cycle. Loss of this effector, which we named ICC1 for inducer of the cell cycle protein 1, produces parasites that are unable to induce a G1/S-phase transition in its host cell. RNAseq analysis has shown that ICC1 is responsible for modulating the expression of a multitude of host genes that are involved in the progression of the host cell cycle into S-phase. We further validate that the presence of ICC1 specifically induces host cell cyclin E which is important for this transition. Overall, our data has elucidated an important role for a *Toxoplasma* dense granule effector in altering the host cell cycle.

P9. Mutations in *Plasmodium falciparum* Pro-drug Activation and Resistance Esterase Mediates Resistance to a Sub-class of Sesquiterpene Dimer Antimalarial Natural Products

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Plasmodium falciparum is the deadliest of the *Plasmodium* species that cause human malaria, and the emergence of drug resistant parasites is a constant threat. Many parasitic mechanisms of drug resistance to antimalarials have been both observed in nature as well as demonstrated in vitro. Recently, *P. falciparum* Prodrug Activation and Resistance Esterase (PARE) was identified and its deactivation implicated in conferring *P. falciparum* in vitro resistance to antimicrobial pepstatin esters as well as the benzodiazepine, MMV011438. Our work, suggests *Pf*PARE deactivation is also a resistance mechanism to the potent antimalarial ester-containing sesquiterpene dimers: Fortunilide A, Fortunilide E and Chlorajaponilide C. In addition, we screened the open source compound libraries called the "Malaria box" and "Pathogen box" using our mutant resistant cell line. Among these libraries, MMV011438 and MMV011576 presented reduced efficacy in parasites resistant to the sesquiterpene dimers. Using a combination of in vitro cell-based assays with parasites harboring mutations in PfPARE gene, recombinant PfPARE enzymatic assays and whole genome sequencing and analysis, we provide evidence that mutations in PfPARE confer resistance to the sesquiterpene dimers. Ex-vivo efficacy studies performed suggest this parasitic mechanism of resistance to several different classes of small molecules is not currently found in field isolates. Analysis of structure-resistance relationships of 11 lindenane type sesquiterpene dimers provides insights for possible future development of these compounds as an antimalarial therapy aimed at preserving low nanomolar efficacy, high selectivity and circumvent possible *Pf*PARE mediated resistance.

P10. Mitochondrial Calcium Homeostasis and Apoptosis in the Protozoan *Crithidia fasciculata*

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The mitochondrial calcium uniporter (MCU) and Bax-1 inhibitor (B1-I) are involved in calcium homeostasis and regulation of cell death. MCU is a transmembrane protein that facilitates calcium uptake into mitochondria, and B1-I participates in calcium homeostasis and mediating cell death in response to stress conditions. While similarities between mammalian and select trypanosome MCU's and B1-I's have been proposed, the role of both proteins in *Crithidia fasciculata* remain largely unexplored. This study establishes that two uncharacterized proteins (ORF8708 and ORF7782) of *C. fasciculate* are homologs of MCU and B1-I, respectively, and that both proteins localize to the mitochondrion. BLASTp, homology modeling, and conserved domain analysis indicate similarity to proteins in other trypanosomatids. Mitochondrial localization was predicted with TargetP and PredSL, and predictions and confirmed by immunofluorescence microscopy in transfects expressing GFP-tagged proteins. Confirmation of a mitochondrial localization sets the stage to assess how each protein contributes to cell death via calcium homeostasis in this protozoal parasite. A large proportion of proteins predicted from genome sequencing are conserved but have no known functions, and our analysis of two of these proteins in *C. fasciculata* illustrates a pathway whereby we can gain a greater understanding of cell biology in pathogens such as *Trypanosoma brucei* and *Leishmania*.

P11. Putative Lysosomal Chloride Channels in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular eukaryotic parasite that invades a wide range of mammalian hosts. In order to invade, the parasites secrete proteins from the microneme, a unique organelle in *Toxoplasma*. Before micronemal proteins arrive at microneme, some of them experience the proteolytic cleavage within the parasite's endolysosomal system for optimal efficiency. A previous study has revealed that the late endosome (LE) is the place where micronemal proteins meet the maturase, a cathepsin L-like protease (TgCPL), for trimming. However, the TgCPL self-maturates within the vacuolar compartment (VAC), a lysosome-equivalent structure, in the parasites. Only a minute amount of TgCPL shuffles from the VAC to the LE to conduct micronemal maturation. Since the activity of TgCPL is pH-dependent, the precise control of pH within parasite's endolysosomal system is crucial. To help acidification of the acidic organelles, the anion channel is needed to help neutralize the accumulation of positive charge incurred by proton pump. Therefore, we speculate that the endolysosomal anion channels are involved in parasite invasion. By homolog search, we identified two putative lysosomal chloride channels by using human lysosomal transporter as a sequence template. These two channels TGGT1_265500 and TGGT1_290330 (TgClC1 and TgClC2, respectively) were endogenously tagged with epitope tag by using the latest genome-editing tool, CRISPR-Cas9. The immunofluorescence assay determined that the TgClC2 localizes in the LE exclusively, while the TgClC1 exists in both of the LE and VAC. I have successfully deleted the TgClC2 in Toxoplasma. Currently, I am conducting a series of phenotypic studies to determine whether the loss of an LE-associated chloride channel can reduce microneme secretion and parasite invasion. Understanding of how *Toxoplasma* regulates pH within its endolysosomal system may provide a new therapeutic strategy for toxoplasmosis.

P12. New Clues about Ivermectin and Its Mode of Action

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In 2016, nearly 800 million tablets of ivermectin were distributed to countries for use in elimination programs for human filarial diseases. Despite its widespread use, the mode of action of ivermectin against filarial nematodes is not well understood, and its in vivo potency cannot be replicated in vitro. To better understand how ivermectin affects filarial worms, our lab previously performed a transcriptomics study to identify differently expressed genes (DEG) in *B. malayi* adults and microfilariae after treatment of infected gerbils. This identified 44 DEG that also had *C. elegans* orthologs available as mutant strains through the *C. elegans* Genetics Center. In this study, we have assayed these mutant strains for differential sensitivity to ivermectin by measuring three phenotypes affected by ivermectin: egg production, development, and motility. We have identified several resistant and hypersensitive strains of *C. elegans* as well as differences between responses to the three assays. Mutations conferring resistance included those in *aff-1*(tm2214), a gene involved in cell fusion events in L4 larvae, and *che-12*(e1812), a gene involved in chemotaxis, cilium assembly, and hyperosmotic response. Hypersensitive strains included RB2287 (*lips-7*), which is predicted to have deficient lipid hydrolase activity. BC177 (*unc-22*) was resistant in both the egg laying and motility assays, but hypersensitive in the development assay. Strikingly, mutations in several genes affecting muscle function caused resistance in the motility assay; expression of all these genes was only altered in drug-treated adult male parasites. Overall, at least 18 genes have been identified as altering ivermectin sensitivity in at least one assay, supporting the validity of the overall approach. These genes may give insight into how ivermectin acts against filarial parasites; we will test the effect of RNAi for these genes on ivermectin sensitivity of *B. malayi* to confirm this.

P13. Mother Does Not Always Know Best: Effects of Methylmercury on Mosquito Oviposition Behavior

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Animals can modulate their own exposure to environmental contaminants through behavioral plasticity such as diet and habitat choice. However, it remains unclear if behavior also has cascading effects on contaminant exposure across multiple generations. In insects, oviposition site selection is an important behavior females can use to modify offspring contaminant exposure risk. In this study we use the yellow fever mosquito, *Aedes aegypti*, to test how methylmercury (MeHg) affects oviposition site selection. We found that mosquito larval development rate and survival were negatively affected at MeHg concentrations ≥ 100 ppb. Adult females not exposed to MeHg as larvae avoided oviposition sites with high MeHg concentrations (> 50 ppb), but MeHg exposure at the larval stage significantly affected this oviposition site selection. Specifically, females raised from larvae exposed to non-toxic MeHg levels (i.e., five-50 ppb) showed a significant increase in preference for oviposition sites contaminated with toxic MeHg concentrations (≥ 500 ppb), compared to unexposed controls. This maladaptive behavioral response could be because, when conditioned with non-toxic MeHg concentrations, MeHg-associated olfactory cues act as a “supernormal” stimulus during oviposition site selection. Importantly, however, this maladaptive behavioral response is eliminated in female mosquitoes raised from larvae exposed to toxic MeHg concentrations (i.e. 100 ppb), and these mosquitoes showed a significant increase in preference for MeHg uncontaminated oviposition sites, compared to unexposed controls. Thus, in mosquitoes, the magnitude of MeHg exposure in one generation can impact MeHg exposure in subsequent generations by altering oviposition site selection behavior. Our results have broad implications for our understanding of how contaminant-mediated behavioral modifications can feedback on contaminant exposure risk across multiple generations, and consequently how behavior can affect the evolutionary trajectory of organisms inhabiting a heterogeneously contaminated environment.

P14. Aptamers as Biosensing Molecules for Malaria Drug Discovery

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Malaria, a mosquito-borne infectious disease caused by *Plasmodium* parasites, is responsible for almost half a million deaths every year. There is an urgent need to find novel drugs and drug targets against the *Plasmodium* parasite to fight the emerging resistance to treatments. DNA aptamers can serve as useful tools in drug discovery by helping to reduce the length of drug screening assays while simultaneously increasing the amount of information that the assays provide about a compound’s toxicity. DNA aptamers are short, single stranded oligonucleotides capable of recognizing a target with high specificity and affinity making them, among other things, effective biosensing molecules. In the present work, we are selecting for a DNA aptamer that recognizes surface markers on infected red blood cells that have been treated with a drug. Serving as a fluorescent reporter molecule, the aptamer binds to these targets and identifies antimalarials in a highly specific and efficient manner. The aptamer was generated by the Systematic Evolution of Ligands by Exponential enrichment technique (SELEX) using artemisinin treated parasites as the positive selection target and both red blood cells and asynchronous cultures in negative selection cycles to increase specificity. This work introduces a novel, expeditious method of identifying stage specific antimalarials and highlights their potential to be used in many aspects of malaria research including therapeutics, diagnostics, and molecular biology.

P15. Metabolic Dependency of Chorismate in *Plasmodium falciparum*

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Plasmodium falciparum is the deadliest of the *Plasmodium* species that cause human malaria, and the emergence of drug resistant parasites is a constant threat. Metabolic pathways have been a source of druggable targets to fight infectious diseases, especially when pathways or their enzymes are sufficiently different or absent in the human host. The shikimate pathway is responsible for the production of chorismate, a branch point metabolite and it is absent in humans. In *Plasmodium*, chorismate is postulated to be a direct precursor in the synthesis of *p*-aminobenzoic acid (folate biosynthesis), *p*-hydroxybenzoic acid (ubiquinone biosynthesis), menaquinone, and aromatic amino acids. While the potential value of the shikimate pathway as a drug target is debatable, the metabolic dependency of chorismate in *P. falciparum* remains unknown. Current evidence suggests that the main role of chorismate is folate biosynthesis despite ubiquinone biosynthesis being active and essential in the malaria parasite. Our goal in the present work was to expand our knowledge of the ubiquinone head group biosynthesis and its potential metabolic dependency on chorismate in *P. falciparum*. These data led us to further characterize the mechanism of action of MMV688345, a compound from the open-access “Pathogen Box” collection from Medicine for Malaria Venture. We systematically assessed the development of both asexual and sexual stages of *P. falciparum* in a defined medium in the absence of an exogenous supply of chorismate end-products and present biochemical evidence suggesting that the benzoquinone ring of ubiquinones in this parasite may be synthesized through a yet unidentified route.

P16. Screening for Specific Inhibitors of the *Plasmodium* Protease, ClpP

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Plasmodium falciparum, the causative agent of malaria, contains a group of parasitic genes that come from prokaryotic origins known as the Clp family proteins. One of these proteins, the ClpP protease, localizes to the metabolic center of the parasite; the apicoplast. While little is known about the apicoplast, it has been shown that drugs that target this non-photosynthetic plastid are clinically effective. The ClpP protease has been shown to be essential to the asexual blood stages of the parasite by forming a complex with other proteins to regulate cellular processes through the degradation of proteins. Through using genetic approaches, a conditional mutant was generated in order to knock down protein levels of ClpP in the parasite by over 95%. Using this cell line as a tool, small molecules from chemical libraries such as the Pathogen Box, a small library of 400 compounds, developed by the Medicines for Malaria Venture, are screened against parasites. Compounds from this library are known to be effective against multiple infectious diseases such as malaria, tuberculosis, and dengue fever, but many of their targets are unknown. It is hypothesized that when knockdown is achieved, the small molecules that are specific to the ClpP protease will be more effective at killing the parasites than when knockdown is not present. With this method, several chemical compounds have been found to potentially be specific to this bacterial protease and these compounds are now being validated.

P17. CBL0137, a Drug Lead for Human African Trypanosomiasis, Perturbs Proteostasis in *Trypanosoma brucei*

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CBL0137, is an orally bioavailable lead drug for Human African Trypanosomiasis (HAT), caused by the eukaryote pathogen *Trypanosoma brucei*. To define modes of action of the compound, we first performed a pharmacokinetic study, giving mice a curative dose (40 mg/kg). Cmax of CBL0137 was 1.841 µM and T1/2 was 6 h. The FreeCmax (concentration of unbound drug at Cmax) was 741 nM. Using axenically cultured trypanosomes, we found that CEC90 (i.e., 90% cidal concentration) is 725 nM. To discover proteins that form complexes with CBL0137, a trypanosome lysate was applied to a CBL0137-agarose affinity matrix, polypeptides were eluted with the drug, and identified by mass spectrometry. Twenty-six proteins selectively bound the affinity column, compared to control agarose beads. To detect in vivo interactions of CBL0137 with the proteins obtained by affinity chromatography, Tb427tmp.01.0870 (RPA1) and Tb427.03.1010, and Tb427tmp.160.4770 as control were tagged with a V5 epitope. Cellular levels of the three proteins were quantitated before and after a 6-h treatment with CBL0137 (725 nM). The steady-state level of all three proteins was reduced up to 60% after 6-h incubation of trypanosomes with CBL0137. We infer that CBL0137 perturbs homeostasis of the tagged proteins. Since RPA1 stimulates DNA synthesis, we hypothesized that its loss from *T. brucei* might affect replication of DNA in the parasite. Consistent with this concept, labeling of DNA with EdU was inhibited after a 3-h treatment of trypanosomes with CBL0137. Thus, CBL037 inhibits DNA synthesis when added a concentration higher than needed to block mitosis (200 nM per 24 h). In a mouse model of HAT, the curation effect may be caused by proteostasis disruption that leads to inhibition of mitosis and DNA replication. Studies are in progress to determine of proteostasis pathways disrupted by CBL0137as part of its mode of action.

P18. Functional Classification of Genes Involved in Mitochondrial Genome Inheritance in the African Trypanosome

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In *Trypanosoma brucei* the mitochondrial genome is organized into a single nucleoid – the kinetoplast. A long-term goal in the field is description of the molecular mechanisms of kinetoplast duplication and inheritance. Conceptually, biogenesis of kinetoplasts has five steps: (i) kinetoplast DNA (kDNA) synthesis; (ii), selection of a site of kinetoplast cleavage; (iii) scission of kDNA; (iv), separation of cleaved kinetoplasts; and (v) partitioning of kinetoplasts into two cells at cytokinesis. Synthesis of kinetoplast DNA (kDNA) has been studied extensively. However, genes involved in other steps of the kinetoplast cycle have not been identified, and we sought to address the topic with genetic analysis. By considering steps involved in producing two kinetoplast networks from a template containing two equivalents of kDNA, and using the five steps in kinetoplast biogenesis (above), we predicted phenotypic changes in the kinetoplasts of trypanosomes with genes mutated at each stage of the cycle. These predictions were compared to primary phenotypes obtained after knockdown of genes associated with kinetoplast inheritance, to arrive at four functional gene sets. Example results are: (i) synthesis of kDNA (e.g., Topo2mt, Pol1B, TbKAP6); (ii) selection of a cleavage site (e.g., TAC p166, TAC65, acyl carrier protein (ACP)); (iii) division (i.e., scission or separation of cleaved kinetoplasts) (e.g.,TbHsIVU, TbCK1.2, KMP-11, TbCEP57); and (iv) partitioning of kinetoplasts into daughter trypanosomes (e.g., TAC102, alpha-KDE2, PNT1, TAC40). This data demonstrates the power of total kinetoplast phenotype analysis in predicting the functions of proteins involved in the biogenesis and inheritance of kinetoplasts. Methods used in this study are applicable to kinetoplasts in other trypanosomatids.

P19. Histidine Ammonia-lyase and Its Role on pH Homeostasis in *Trypanosoma cruzi*

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Amino acids constitute a carbon, nitrogen and energy source in trypanosomatids. In *T. cruzi*, L-histidine can be deaminated into urocanate by the histidine ammonia-lyase (TcHAL) [EC 4.3.1.3] with concomitant production of ammonia (NH3). This latter is converted into ammonium ($NH4^{+}$) under physiological pH. Subcellular fractionation, western blot and immunofluorescence analyses showed that TcHAL is localized to the acidocalcisomes. To determine a possible function for this basic amino acid within these acidic compartments, we analyzed changes in pH driven by L-histidine. Fluorometric measurements using acridine orange in isolated acidocalcisomes showed increases in pH triggered by histidine addition. Also, ammonium production from histidine was detected in these organelles, thus suggesting that histidine deamination alkalinizes acidocalcisomes. Recent affinity purification studies from our lab (Negreiros et al., *Mol. Microbiol*., 2018) identified TcHAL as a polyP-binding protein, and we aimed at validating such interaction. Kinetic studies of TcHAL from parasite lysates showed a 2-fold reduction in enzymatic activity in the presence of polyP100. Analysis of recombinant TcHAL-6xHis and TcHAL-3xHA from parasite lysates showed differences in mobility after polyP addition or exopolyphosphatase (PPX1) treatment. Primary sequence of TcHAL revealed the presence of a putative PASK-like domain, which has been reported as a protein signature for protein polyphosphorylation. Using CRISPR/Cas9-mediated endogenous C-tagging, we generated knock in parasites ($TcHAL^{K-L}$-3xHA) harboring specific mutations (K525F, K526L, K531L and K533L) in that PASK-like domain present at its C-terminus. These $TcHAL^{K-L}$cells displayed defects in enzyme activity, and cellular localization and failed to differentiate into metacyclic trypomastigotes when histidine was present. Our data suggest that TcHAL can be involved in pH regulation of acidocalcisomes through its interaction with polyphosphate, a process exclusive to *T. cruzi*.

P20. *Trypanosoma cruzi* Possesses Four Different Mitochondrial Calcium Uniporter (MCU) Ca^{2+}-Conducting Subunits Important for Host Infection and Replication

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Trypanosoma cruzi, the etiologic agent of Chagas disease, possesses a mitochondrial calcium uniporter (MCU) complex with physiological characteristics similar to those of the mammalian one. However, we report here that *T. cruzi*, as well as other trypanosomatids, possess 2 unique paralogs of the MCU subunit that we named *TcMCUc*, and *TcMCUd*. The predicted structure of the proteins indicates that, as that predicted for the *TcMCU* and *TcMCUb* paralogs, they are composed of two helical membrane-spanning domains, and contain a WDXXEPXXY motif, suggesting that each one of the four paralogs could form part of the pore in a hetero-oligomeric MCU complex. Overexpression of each gene led to a significant increase in mitochondrial Ca^{2+} uptake. Using the CRISPR/Cas9 technique we obtained knockouts (KO) of *TcMCUc* and *TcMCUd* leading to a loss or significant decrease of mitochondrial Ca^{2+} uptake, respectively, without affecting the mitochondrial membrane potential. *TcMCUc*-KO and *TcMCUd*-KO epimastigotes exhibited reduced growth rate in low glucose medium and alterations in their respiratory rate, citrate synthase activity and AMP/ATP ratio, while trypomastigotes had reduced ability to efficiently infect host cells and replicate intracellularly as amastigotes. By gene complementation of KO cell lines or by performing knock-ins using a CRISPR/Cas9-based methodology we also studied the importance on mitochondrial Ca^{2+} uptake of critical amino acid residues within or near the WDXXEPXXY motif of the four paralogs. In conclusion, the results predict a hetero-oligomeric structure for the *T. cruzi* MCU complex, with structural and functional differences, as compared to those in the mammalian complex.

P21. Characterization of a Phospholipase C-like Protein (TbPI-PLC2) from *Trypanosoma brucei*

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Trypanosoma brucei is the causative agent of African Trypanosomiasis, a deadly disease affecting humans and cattle. There are very few drugs to treat the disease and evidence of mounting resistance raises the need for new drug development. The inositol 1,4,5 triphosphate/diacylglycerol (IP3/DAG) pathway regulates important processes in many organisms. *T. brucei* has an active IP3 receptor, localized to the acidocalcisome, that is essential for infection in mice. In previous work (King-Keller et al., *Eukaryot. Cell* 14:486-494, 2015) we characterized a phosphoinositide phospholipase C (TbPI-PLC1, Tb927.11.5970) from *T. brucei* that contains a domain organization characteristic of PI-PLCs, such as X and Y catalytic domains, an EF-hand calcium-binding motif, and a C2 domain, but lacks a pleckstrin homology (PH) domain. In addition, TbPI-PLC1 contains an N-terminal myristoylation consensus sequence only found in trypanosomatid PI-PLCs. Here we report the presence of a second PI-PLC-like protein (TbPI-PLC2, Tb927.6.2090) that is very similar to TbPI-PLC1 but lacks the Y catalytic domain and the C2 domain and possesses instead a PDZ domain. Recombinant TbPI-PLC2 hydrolyzes neither phosphatidylinositol (PI) nor phosphatidylinositol 4,5-bisphosphate (PIP2), and does not modulate TbPI-PLC1 activity. However, knockdown of *TbPI-PLC2* expression alone or together with downregulation of *TbPI-PLC1* expression by RNAi resulted in growth inhibition. This is in contrast with the lack of effect of downregulation of expression of *TbPI-PLC1* alone. TbPI-PLC2 has a plasma membrane and intracellular localization and it might be involved in IP3 binding as has been reported for the phospholipase C-related catalytically inactive protein 1 (PRIP-1) of mammalian cells (Uji et al., *Life Sci* 72:443-453, 2002). The PDZ domain could be involved in this binding and this is being investigated.

P22. *Brugia malayi* microRNAs and Potential Targets within the Feline Host (*Felis catus*)

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Host specificity is a fundamental concept in the survival and growth of parasites within a host. In host-parasite relationships, many mechanisms have been documented by which parasites, notably helminths, modulate the immune system of their host to promote their survival. Due to the evolutionary history of these relationships, hosts often become disease-tolerant or asymptomatic, even with high worm burdens, until there is a dysregulation in the host immune system that can induce a disease state. The molecular mechanisms of how parasites modify the host immune system and the host environment as a whole are not fully understood. What is generally accepted is that parasitic nematodes release a variety of excretory/secretory (E/S) products that have been found to enable the worms to safely penetrate and migrate through host tissues while eliciting little to no host immune response. These E/S secretions have been found to secrete microvesicle and exosomes that contain small RNAs such as microRNAs (miRNA). MicroRNAs have been extensively studied and observed to induce a number of disease states in mammalian hosts along with modulation towards a tolerant environment for parasite proliferation. To investigate this relationship, we analyzed plasma of four *Brugia malayi*-infected cats with varying microfilaremias six months post-infection. From the ~32 million sequencing reads, 185 mature miRNA sequences of potential *B. malayi* origin were detected in feline plasma, with 26 miRNAs present in 10 copies or more. By in silico methods, 7 immune genes (Ptgs1, Irf4, Irf5, Numbl, Tnfsf15, Stat3, and Txlnb) were identified as high-confidence predicted targets of parasite-derived miRNAs on the feline genome. These miRNAs could be manipulating the host immune systems which would implicate a potential molecular mechanism in host immune system down-regulation.

P23. Regulation of Physiology and Organellar Organization within the *Toxoplasma* Endolysosomal System

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Toxoplasma gondii is an obligate intracellular protozoan parasite, which currently infects approximately one-third of the human population. The lysosome-like vacuolar compartment/plant-like vacuole (VAC/PLV) is an important organelle within *T. gondii*, that houses proteolytic enzymes which rely on an acidic pH in order to function properly. The endolysosomal system within *T. gondii* parasites includes organelles such as the VAC as well as the early and late endosomes, currently recognized collectively as the endosome-like compartment (ELC), which are involved in the endocytosis and digestion of host proteins. We have observed an aberrant overlap of the swollen VAC and the ELC within a mutant strain of parasites lacking the VAC-residing transmembrane chloroquine resistance transporter (CRT; Δ*crt*), which was not observed in the WT and complement (*ΔcrtCRT*) strains. This aberrant organellar co-localization is likely to alter the physiology of the VAC and ultimately inhibit proper function of the organelle. To better define the molecular mechanism by which TgCRT affects the organellar organization within the endolysosome in *Toxoplasma* parasites, we have cloned and fused a 3xHA epitope tag at the N-termini of the classic early and late endosome organelle markers, TgRab5 and TgRab7, for future high-resolution microscopy. Furthermore, quantitative PCR and endogenous epitope tagging revealed that four proteases residing within the endolysosomal system displayed down-regulated transcripts and/or decreased protein levels in *Δcrt*, further indicating that VAC physiology is altered. We also introduced the pH-sensitive GFP biosensor, pHluorin2, into *Toxoplasma* to quantify both the cytosolic and the vacuolar pH within WT, *Δcrt*, and *ΔcrtCRT* parasites. Collectively, our work will contribute to a better understanding of how *Toxoplasma* parasites regulate the dynamic process of separating endolysosomal vesicles in order to maintain infectivity as well as preserve optimal physiology within the lysosome-like VAC.

P24. Characterization and Inhibition of Protoporphyrinogen IX Oxidase (PPO) in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis. Toxoplasmosis can be fatal to the immunocompromised population. Exploration of differences in the nutrient metabolism pathways between the host and *T. gondii* will lead to better clinical treatments for toxoplasmosis. *T. gondii* encodes an intact heme biosynthesis pathway. However, the extent to which *Toxoplasma* uses its de novo heme biosynthesis to support its growth remains unclear. In this study, we genetically ablated protoporphyrinogen IX oxidase (TgPPO) in the parasites, which catalyzes the second last reaction within the heme biosynthesis pathway. We determined the total heme content in TgPPO-deficient mutant (Δ*ppo*) was reduced by ~50% compared to wild type (WT) parasites. Additionally, the Δ*ppo* strain exhibited significant defects in plaque formation and intracellular replication. Strikingly, the TgPPO-deficient parasites lost their virulence in a murine model. Given that TgPPO significantly affects the pathogenesis of *T. gondii* and low-toxicity PPO inhibitors are widely used in weed control. We tested 11 commercially available PPO-targeting herbicides on the growth of WT *T. gondii* using a luciferase-based growth assay. Of these 11 inhibitors, oxadiazon, oxyfluorfen, fluthiacet-methyl, and lactofen have been determined to significantly decrease *T. gondii* growth. This study, in conjunction with future work testing the specificity of the inhibitors and their effectiveness in vivo, will establish a proof-of-concept that inhibition of TgPPO is a potential therapeutic strategy in managing toxoplasmosis.

P25. Scission of Mitochondrial Nucleoid is Regulated by a Protein Kinase in the African Trypanosome

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The single mitochondrial nucleoid (kinetoplast) of *Trypanosoma brucei* is essential for viability of the parasite. Consisting of interlocked circular DNAs, kinetoplast DNA (kDNA) synthesis is completed and the network divided into two daughter networks before it is partitioned into daughter cells. Basal bodies, microtubule organizing centers for flagella, are found near kinetoplasts, and their separation is associated with division of kinetoplasts. Whereas significant progress has been made in our understanding of how kDNA is synthesized, and many genes involved in kinetoplast inheritance are known, genes that are essential for division (i.e., cleavage/scission and separation) of kinetoplasts have received little attention. Knockdown of a protein kinase TbCK1.2 inhibits division of kinetoplasts (K) but nuclear (N) mitosis is unaffected, resulting in mutant 1K2N trypanosomes. In these 1K2N cells, separation of basal bodies is normal, and kDNA synthesis is completed. Thus, basal body separation does not cause division of kinetoplasts. On account of this and other data, we posit existence of a short-lived intermediate in division of kinetoplasts where the nucleoid is flanked by two well-separated basal bodies. This post-basal body separation intermediate accumulates when genes required for division of kinetoplasts (i.e., Kinetoplast Division Factors (KDFs)) are knocked down. Functions of KDFs are distinguishable from proteins associated with a tripartite attachment complex (TAC) region. We developed five phenotypic criteria to identify KDFs, and found 14 candidates in the literature. We show that TbCK1.2 and TbBBP46 meet initial tests as KDFs. Strikingly, uncleaved kinetoplasts, up to three times normal length, accumulate after knockdown of TbCK1.2, providing the first evidence in any biological system for protein kinase regulation of mitochondrial nucleoid scission.

P26. MICU1 and MICU2 Play an Essential Role in Mitochondrial Ca^{2+} Uptake, Growth and Infectivity of the Human Pathogen *Trypanosoma cruzi*

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The mitochondrial Ca^{2+} uptake in trypanosomatids, which belong to the eukaryotic supergroup Excavata, shares biochemical characteristics with that of animals, which, together with fungi, belong to the supergroup Opisthokonta. However, the composition of the mitochondrial calcium uniporter (MCU) complex in trypanosomatids is quite peculiar, suggesting lineage-specific adaptations. In this work we used *Trypanosoma cruzi* to study the role of orthologs for mitochondrial calcium uptake 1 (MICU1) and MICU2 in mitochondrial Ca^{2+} uptake. TcMICU1 and TcMICU2 have mitochondrial targeting signals, two canonical EF-hand calcium binding domains, and localize to the mitochondria. Using the CRISPR/Cas9 system, we generated *TcMICU1* and *TcMICU2* knockout cell lines. Ablation of either *TcMICU1* or *TcMICU2* showed a significantly reduced mitochondrial Ca^{2+} uptake in permeabilized epimastigotes without dissipation of the mitochondrial membrane potential or effects on the AMP/ATP ratio or citrate synthase activity. However, none of these proteins had a gatekeeper function at low [Ca^{2+}], as occurs with their mammalian orthologs. *TcMICU1*-KO and *TcMICU2*-KO epimastigotes had a lower growth rate and impaired oxidative metabolism, while infective trypomastigotes have a reduced capacity to invade host cells and to replicate within them as amastigotes. The findings of this work, which is the first to study the role of MICU1 and MICU2 in organisms evolutionary distant from animals, suggest that, although these components were probably present in the last eukaryotic common ancestor (LECA), they developed different roles during evolution of different eukaryotic supergroups. The work also provides new insights into the adaptations of trypanosomatids to their particular life styles.

P27. Molecular Study of Axonemal Dynein's Role in the Unique Flagellar Bend Propagation in *Trypanosoma brucei*

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The flagellar motility of kinetoplastids is essential for multiple aspects of their cell biology, including viability, virulence, transmission and cell morphology. The flagellar beating is powered by an axoneme decorated with team of dyneins and exhibits a unique bending wave that propagates from the tip to the base rather than base-to-tip, as all other eukaryotes do. We hypothesize that tip-to-base beating is due to unique biophysical spatiotemporal coordination mechanisms of kinetoplastid axonemal dynein motor proteins acting in teams. We are purifying axonemal dynein from *T. brucei*, a unicellular kinetoplastid that causes lethal sleeping sickness disease in human, to make single-molecule measurements of its fundamental biophysical properties. We used RNA interference to knockdown flagellar attachment proteins to enable isolation of the flagellum from the cell bodies by mechanical shearing and differential centrifugation. We also constructed a biotin-binding (BCCP) domain, His6 tag, and eGFP labeled version of LC2 (light chain on outer-arm dynein) and induced *T. brucei* to express it to facilitate dynein purification and use of that dynein in subsequent biophysical assays (binding of dyneins to microspheres for tweezer assays or visualization of fluorescently-labeled dynein in microscopy). We aim to make direct measurements of single dynein motor undergoing its power-stroke and quantify the force dependence of dynein's biophysical properties including microtubule binding and unbinding rates, step size, power-stroke and speed. We expect that these results will provide critical insight into the molecular mechanisms of the unique tip-to-base beating in kinetoplastids and ultimately serving as potential drug targets against kinetoplastid diseases.

P28. Catch Me If You Can: Identifying Fast-evolving Genes in the Genus *Cryptosporidium*

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Cryptosporidium is a widespread apicomplexan parasite that causes cryptosporidiosis, the second leading cause of diarrheal disease in infants globally. It is often fatal in immunocompromised patients. This genus has at least 30 different characterized species with a broad host range. Unfortunately, single locus genotyping of just one marker *gp60*, which encodes the highly polymorphic 60 kD glycoprotein (GP60), is used as the standard for intraspecific genotyping. This marker indeed parallels some phenotypic traits, like host preference, but like many other parasites, *Cryptosporidium* reproduces sexually during its life cycle leading to frequent genetic recombination, yielding increased genetic diversity between samples. Thus, a single-locus is unable to correctly represent population structure. The goal of this project is to identify other potentially fast-evolving loci that can be used for multilocus subtyping of *Cryptosporidium*. All proteins are susceptible to evolutionary pressures. Depending on the nature of these forces, genes can undergo positive, negative, and neutral selection. This project is focused exclusively on genes undergoing positive selection, presumably through the pressure of the host immune system. To identify positively selected genes, variations in single nucleotide polymorphisms (SNP's) were identified in divergent *Cryptosporidium* genome sequences. The SNP data, when combined with genome annotation, permits evaluation of synonymous and non-synonymous polymorphisms, which can in turn be used for dN/dS calculations. Identification of additional fast-evolving gene candidates through dN/dS analysis will help determine loci that may be useful for multilocus genotyping classification.

P29. Calcium Signaling and *Toxoplasma* Motility

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Calcium signaling is utilized universally across life, as binding of Ca^{2+} to signaling effectors induces a cascade of downstream processes. Fluxes in basal cytosolic Ca^{2+} levels ($[Ca^{2+}]i$) serve as the basis for signaling, as mechanisms to increase or decrease cytosolic Ca^{2+} are intricately balanced. *T. gondii*'s pathogenesis and lytic cycle are linked, as calcium oscillations originating from extracellular influx and/or release from intracellular stores precede the induction of the lytic cycle processes of motility, invasion, and egress. For a successful lytic cycle, *T. gondii* must traverse biological barriers and invade host cells, and motility is essential for both. Using genetically encoded calcium indicators (GECI's), in particular GCaMP6f (GFP based calcium indicator) expressing parasites and host cells transiently expressing red GECI's, we can track Ca^{2+} dynamics of both the parasite and host cell in real time. Within a host cell *T. gondii* resides within a specialized intracellular vacuole that functions as a sieve to passively permit for the exchange of small molecules; thus, the surrounding milieu of intracellular parasites is likely in equilibrium with the host cytoplasm. Therefore, intracellular parasites are liable to the same fluxes of host cytosolic ionic composition that occur throughout a host cell signaling event. During intracellular growth, an unknown signal induces calcium oscillations that precede motility and egress, yet we believe this Ca^{2+} signal must meet a threshold for egress. Post achievement of a Ca^{2+} threshold, activation of an unknown agonist induces a signaling pathway, resulting in Ca^{2+} oscillations and is enhanced by later downstream extracellular calcium flux, culminating in motility stimulation and egress. By using whole-cell patch clamp, we delivered exact concentrations of $[Ca^{2+}]i$ to the cytosol of infected host, to determine the level necessary for egress. Our data established the role of Calcium influx from the host cytoplasm and its impact on motility and egress.

P30. Characterization of an Oxygen-independent Coproporphyrinogen Dehydrogenase in *Toxoplasma gondii*

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Toxoplasma gondii is an apicomplexan parasite that is the sole cause of Toxoplasmosis, a severe and potentially lethal disease for immunocompromised and pregnant individuals. Due to its parasitic nature, *T. gondii* requires small nutrient molecules synthesized de novo or scavenged from host cells in order to replicate intracellularly. Heme, a vital nutrient molecule, serves as a prosthetic group for many fundamental cellular processes such as electron transport and redox reactions. *T. gondii*'s genome contains all genes necessary for the classic heme biosynthetic pathway and through genetic deletion of these genes, our preliminary data show that de novo heme synthesis is essential for the growth and pathogenesis of *T. gondii*. The antepenultimate step of the classical pathway utilizes the enzyme, coproporphyrinogen oxidase (CPOX), that relies on the presence of oxygen to function. Our preliminary results show that the *Δcpox* strain displays a dramatic reduction in intracellular growth under ambient conditions but still remains viable. This possibly indicates an alternate pathway used for survival. Interestingly, *T. gondii* contains an ortholog of the oxygen-independent coproporphyrinogen dehydrogenase enzyme (CPDH) that has been previously characterized in the anaerobic heme biosynthetic pathway in prokaryotes. RNA-seq data reveals that there is an increase in TgCPDH transcripts within the *Δcpox* strain. In addition, *Δcpox* parasites have improved intracellular growth under hypoxic conditions. Based on these findings, we hypothesize that the parasites may switch to an alternate oxygen-independent pathway in the absence of TgCPOX by utilizing TgCPDH. Collectively, our findings suggest that an alternative heme synthetic pathway may be utilized in vivo and can be targeted as a therapeutic treatment for Toxoplasmosis.

P31. Assembly of a *Dirofilaria immitis* Genome for Multiple Drug-Susceptible and Resistant Strains for Comparative Genomic Analyses

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Dirofilaria immitis, the parasite responsible for heartworm disease in cats and dogs, is perhaps the most impactful parasitic disease to affect cats and dogs in the United States. Although these infections are typically prevented via monthly administration of macrocyclic lactone (ML) drugs to healthy animals, resistant isolates of *D. immitis* have been increasingly reported throughout the past fifteen years. This makes it a high priority for us to develop genetic tools to monitor the prevalence of alleles for ML resistance among *D. immitis* populations in the United States. As an early step in that process, we are currently conducting a comparative genomic analysis of five different strains: two of which have been identified as "susceptible" to ML treatment (Missouri and Georgia-2), and three more as "resistant" (Yazoo-2013, Metairie-2014, and JYD). Our approach to do so is two-fold: First, we have created two de novo assemblies using PacBio sequencing platforms: one for the Georgia-2 strain and another for Yazoo-2013. Each resulting PacBio assemblies for Georgia-2 and Yazoo has returned a 89-Mb genome spanning hundreds of contigs. In turn, we have also generated Illumina sequence reads to support our PacBio base-call correction and help fill in the gaps between the assembled contigs. This two-fold approach will help us generate a hybrid scaffold assembly between both platforms for each strain for methodological comparison. Upon completion of each strain's genome assembly, future experiments will be directed towards performing genome annotations of these strains. These efforts will be focused on evaluating the genomic variation that occurs within and between each strain, enabling us to proceed with the identification of genetic markers in the *D. immitis* genome that may confer to susceptibility or resistance to ML drug treatment.

P32. Molecular Characterization of the Invasive Tick, *Haemaphysalis longicornis*, and Development of a Restriction Fragment Length Polymorphism (RFLP) Assay to Distinguish *Haemaphysalis* Species

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Ticks are important vectors for pathogens of significant public and veterinary health importance. The ixodid tick, *Haemaphysalis longicornis*, endemic to East Asia and invasive in New Zealand and Australia, has recently become established in the eastern U.S. *H. longicornis* is especially concerning due to its ability to reproduce parthenogenically (thus easier for the tick to become established in new regions), the large number of pathogens it transmits, a diverse host range, and the ability to develop very high tick burdens on hosts resulting in losses. Currently, there are four *Haemaphysalis* species in the New World. Adults and nymphs can easily be distinguished morphologically and, although more difficult, larvae can be distinguished as well. However, many features necessary for identification are on the mouthparts which can easily be damaged or lost during collection. Therefore, we developed a restriction fragment length polymorphism (RFLP) assay utilizing the 16S ribosomal RNA and cytochrome c oxidase subunit I (COI) gene regions to differentiate between the invasive and native *Haemaphysalis* spp. In both gene regions, we found that genetically similar species of *Haemaphysalis* have unique RFLP cutting patterns. Thus, using this RFLP assay, we were able to accurately identify and distinguish between *H. longicornis* and other *Haemaphysali*s spp. In order to potentially predict the origin region for the established *H. longicornis* and monitor its spread, we also conducted a phylogenetic study on these sequences. Preliminary results for 16S (n=30) and COI (n=32) have revealed two and three unique lineages of *H. longicornis*, respectively. Overall, our study provides a more rapid and cost-effective method for confirming the presence of this invasive tick and suggests that only limited variation was noted in these two gene targets.

P33. DNA Recombinases, Rad51 and Dmc1, of *Entamoeba*

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Entamoeba histolytica is the causative agent of amoebiasis and liver abscesses, and is prevalent in developing countries. The pathogen infects 50 million people annually. *Entamoeba* species have two-phase life cycles: active, virulent trophozoites and dormant, environmentally-stable cysts. Only cysts are capable of establishing new infections, and are spread by fecal deposition. Rad51 and Dmc1 are DNA recombinases involved in double-stranded DNA repair and meiotic recombination, respectively. Our laboratory has previously shown that *E. histolytica* possesses authentic Rad51 and Dmc1 recombinases, which are capable of facilitating DNA recombination in vitro. However, the roles that they play in vivo have not been fully explored (1, 2). The presence of a meiotic recombinase, such as Dmc1, in *Entamoeba* is particularly intriguing as this parasite does not have an apparent sexual life cycle. To further understand the roles of Rad51 and Dmc1 in vivo, we will create Rad51 and Dmc1 knockdown (KD) cell lines in both *E. histolytic*a (*eh*Rad51-KD, *eh*Dmc1-KD) and *E. invadens* (*ei*Rad51-KD, and *ei*Dmc1-KD) using an RNAi-based method known as the Trigger approach (3, 4). Currently, there are no methods to induce stage conversion in the human pathogen (*E. histolytica*); thus, the reptilian pathogen (*E. invadens*), which readily encysts in vitro, serves as a model. We will characterize virulence and stage conversion in the KD cell lines. Specifically, we will track the rate of homologous recombination, measure a variety of virulence functions such as phagocytosis, host-parasite adhesion, and host cell cytolysis in both species. We will also measure both encystation and excystation rates in *E. invadens* KD mutants. To date, we have successfully subcloned cDNAs encoding Rad51 and Dmc1 into the Trigger plasmids and have begun to create cell lines by transfection.

P34. Host and Environmental Factors Differentially Affect Parasite Community Structure and Infection Dynamics in a Montane Biodiversity Hotspot

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Montane ecosystems, characterized by predictable climatic gradients and unique ecological communities, serve as excellent natural laboratories to test how biotic and abiotic factors interact to affect disease dynamics. Avian malaria, a vector-borne disease caused by protozoan parasites such as *Plasmodium* spp. and *Haemoproteus* spp. is known to infect bird populations globally. Here we examine how host (e.g., phylogeny and ecology) and environmental (e.g., climate and anthropogenic disturbance) factors interact to influence parasite phylogenetic diversity – alpha diversity, parasite community turnover – beta diversity and infection risk among birds inhabiting a montane biodiversity hotspot – Western Ghats, India. We sampled 1177 birds across 28 species and amplified 480bp of parasite mitochondrial cytochrome b gene to characterize avian malaria infection. Our results showed that alpha diversity of host specialist *Haemoproteus* parasites was primarily affected by host phylogenetic diversity whereas alpha diversity of generalist *Plasmodium* parasites was primarily affected by host functional diversity. While *Haemoproteus* beta diversity was strongly influenced by host beta diversity, *Plasmodium* beta diversity was primarily influenced by abiotic differences among the sites (e.g., elevation and precipitation). Furthermore, our results revealed that elevation exerted a significant direct and negative effect on infection risk for both *Haemoproteus* and *Plasmodium*. However, for *Plasmodium*, elevation also exerted a significant indirect and positive effect on infection risk through its effects on other factors affecting infection dynamics (e.g., functional characteristics of the hosts and anthropogenic disturbance). Consequently, our results indicate that climatic, host and anthropogenic factors that covary with elevation can interact in complex ways to alter the underlying elevational gradient of infection risk. Our study highlights the importance of disentangling the direct effects of elevation from other host and environmental variables to improve our understanding of disease dynamics in montane ecosystems.

P35. WD40 is More Than Just a Rust Remover: Novel WD40 Proteins with Potential Roles in Cell Regulation

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Mitochondrial proteins in the WD40 superfamily contribute to a wide range of functions including signal transduction and cell cycle regulation. The roles of WD40 proteins in pathogenesis of *Leishmania* and *Trypanosoma* infections are unclear, and we are analyzing these proteins in *Crithidia fasciculata*, a trypanosomatid that parasitizes flies but does not infect humans. Of the 150 WD40 proteins annotated in the *Crithidia* genome, two uncharacterized members (ORF_3102 and CFAC1_260034800) were identified by conserved domain analysis and BLAST. ORF_3102 contains three C-terminal WD40 domains. Consistent with the prediction to the localization to the endoplasmic reticulum or plasma membrane, this gene encodes a protein with one possible transmembrane segment (PSORT II). The homologue of ORF_3102 in *T. brucei* is constantly expressed throughout the lifecycle (Queiro et al., 2009). In contrast to ORF_3102, CFAC1_260034800 has six WD40 domains and no transmembrane helices. This protein is similar to *Homo sapiens* WD repeat protein 7. Genes for both *Crithidia* proteins will be expressed in *Crithidia* as GFP-fusion proteins using the pNUS-GFPcH vector (Tetaud et al., 2002). Western blot will be utilized to confirm expression, and subcellular localization will be determined by immunofluorescence microscopy.

P36. Population Genomics of the Invasive Tick, *Haemaphysalis longicornis*

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The Asian longhorned tick, *Haemaphysalis longicornis*, is native to eastern Asia, but has invaded Australia, New Zealand, and most recently, the U.S. There are several life history traits of *H. longicornis* that enhance its ability to invade new environments. First, it has a broad geographic and host range including humans, domesticated animals and a wide variety of wild carnivore, cervid, rodent, and avian species. Furthermore, some populations of *H. longicornis* are capable of parthenogenesis. Therefore, a single female tick introduced to a new area can start a new population. Population genetics have been extensively used to investigate arthropod vectors through estimating gene-flow, dispersal patterns and their potential to spread pathogens. For *H. longicornis* specifically, determining the genetic structure of tick populations within the U.S. will potentially elucidate critical questions about this invasive tick such as: i) the number of introductions (or lineages) into the U.S. and, ii) population origin of ticks in the U.S. We obtained restriction-site associated DNA sequences (RADseq) from *H. longicornis* collected from several U.S. states (AR, VA, NJ, NY, and WV) and Australia. We detected 3883 variable loci with 16,168 SNPs among these samples. Our data indicate that *H. longicornis* has modest amounts of variation within collection locations, but moderate to large amounts of variation among locations. Little variation was detected between NY and NJ, as well as between AR and VA. We find four groups (NY+NJ, AR+VA, WV, and Australian) that are quite distinct from each other. Thus, our data demonstrate that there is considerable genetic variation and structure among the U.S. populations of *H. longicornis* and supports the use of this technique to investigate sources for this invasive tick.

P37. The Inositol Polyphosphate Pathway of *Toxoplasma gondii*

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Inositol polyphosphates are a diverse class of intracellular messengers present in eukaryotic cells. Myo-inositol is the basic building block with six possible positions for phosphorylation. The fully phosphorylated form is known as phytic acid. Further addition of diphosphates to the ring results in the production of inositol pyrophosphates. These water-soluble and high-energy small molecules are involved in critical cellular functions such as vesicle trafficking, phosphate homeostasis, ribosome synthesis and stress response. Inositol pyrophosphates play roles in signaling and regulation of gene expression. Inositol polyphosphates pathway has not been studied in *T. gondii*, however genes that encode three of the inositol phosphate kinases of the pathway are present in its genome. We cloned, expressed and purified the recombinant protein of two of these enzymes. Inositol polyphosphate multikinase (TgIPMK), enzyme with dual activity that catalyzes the conversion of inositol trisphosphate (IP3) into inositol-tetrakisphosphate (IP4) and inositol-pentakisphosphate (IP5); and the hexakisphosphate kinase (TgIP6K), that uses inositol-hexakisphosphate (IP6) to produce inositol pyrophosphates. We showed that both enzymes have kinase activity. TgIPMK was able to use IP3 as substrate to produce IP5 and, TgIP6K can use IP5 to produce diphosphoinositol-tetrakisphospahte (PP-IP4) and IP6 to produce 5-diphospho-inositol-pentakisphosphate (5-IP7). We investigated subcellular localization of TgIPMK and TgIP6K by endogenous tagging and found cytosolic localization in extracellular tachyzoites. Furthermore, using the tetracyclin-regulated transactivator expressing strain (TatIΔku80) we developed conditional knockouts of TgIPMK (iΔTgIPMK) and TgIP6K (iΔTgIP6K) and showed that these enzymes are essential for parasite growth. This is the first evidence that protein pyrophosphorilation occurs in *T. gondii*.

P38. Screening for Reversal/Prevention of Dormancy and/or Amastigote to Trypomastigote Stage Conversion in *Trypanosoma cruzi*

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The current screening for compounds against *Trypanosoma cruzi* is focused exclusively on detecting toxicity for actively replicating amastigotes. However the finding of dormant forms of the parasite resistant to trypanocidal drugs emphasizes the need for new strategies to test compounds active against this dormant form. We propose a novel approach to compound screening for *T. cruzi*, searching not for compounds that directly kill parasites within host cells but rather for compounds that prevent or reverse dormancy and/or drive the stage conversion of amastigotes into the non-replicating trypomastigote form. For this purpose we designed an in vitro screening assay with parasites expressing the fluorescent protein Tdtomato to indicate the overall parasite load and allow for the detection of compounds that kill both actively dividing and quiescent parasites; and expressing the fluorescent protein mNeon-NLS-sec as a premature stage transition marker which allows for the identification of compounds that drive amastigote to trypomastigote stage conversion. These transgenic parasites are also stained with the CellTrace Violet dye which is diluted by replication in actively dividing amastigotes but retained in dormant parasites. Significant decreases in the number of dye- positive amastigotes will indicate compounds that either kill dormant parasites, or induce their proliferation/stage conversion. We adapted these cultures to a robotized cell seeding process in a 384 wells plate format for the high throughput screening of compound libraries. The number of infected cells and time of culture have been optimized to provide sufficient numbers of dormant forms per well. The use of frozen host cells and parasites were standardized to ease the transfer of the assay to other screening facilities which will help in the adoption of this technique by the research community and the development of alternative screening approaches that consider the dormant forms of the parasite.

P39. Worms Infecting Worms: Exploring the Remarkable Diversity of Blood Fluke Larval Stages Developing in Polychaetes from the Atlantic Coast of South Carolina

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Molluscs are intermediate hosts of most digeneans, whereas polychaetes are intermediate hosts for some suspected fish blood flukes. For many years this interesting divergence of blood fluke development has been underexplored. Recently we've reported life cycles of *Cardicola laruei* and *C. parvus* (see Siegel et al., 2018) as well as the unexpected finding that *Neospirorchis* spp. (turtle blood flukes) develop in polychaetes (de Buron et al., 2018). During the collections of specimens for the aforementioned reports, we found five additional undescribed blood fluke larvae. These include another *Cardicola* sp. with cercariae in sporocysts found in both *Enoplobranchus sanguineus* and *Amphitrite ornata* as well as a mixed infection with *Neospirochis* sp. in *E. sanguineus*; sequence data of ITS2 are most closely related to *C. aurata* (93% similarity). We found another *Cardicola* sp. in *A. ornata* that has 94% ITS2 sequence similarity to *C. milleri*. A third *Cardicola* sp. found in *Loimia* sp. had microcercous cercaria, similar in morphology to *C. orientalis*, and 94% similarity (ITS2) to *C. forsteri*. Of great interest were rediae of two species in *E. sanguineus*, one with furcocercous cercariae with body fin (no molecular data) and one with 95% sequence similarity to P*settarium nolani* (no cercariae observed); these latter findings further expands the role of polychaetes in life cycles of blood flukes. In summary, our studies of digenean larval stages in polychaetes reveal a remarkable diversity of blood flukes that utilize the same annelids as intermediate hosts.

P40. Characterization of the *Naegleria fowleri* Glucokinase: Is Disruption of Glucose Metabolism a Viable Therapeutic Approach?

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The free-living amoeba, *Naegleria fowleri*, is the causative agent of primary amoebic meningoencephalitis (PAM), a rare yet usually lethal infection of the brain. In an effort to identify new targets for therapeutic intervention, we have scored the importance of glucose metabolism. Supporting the suitability of this pathway for target identification, *N. fowleri* trophozoite growth is severely impaired when cultured without glucose. The *N. fowleri* genome encodes a glucose phosphorylating enzyme, a glucokinase (NfGlck), that generates glucose-6-phosphate, an important intermediate for both glycolysis and the pentose phosphate pathway. Following cloning and heterologous expression, NfGlck was found to be a monomer with an apparent molecular mass of 47.7kDa. The enzyme had apparent Km values of 42.5 ± 7.3 µM and 141.6 ± 9.9 µM for glucose and ATP, respectively, and the structure of the enzyme in complex with the ATP analog AMPPNP and G6P has been resolved to 2.2Å (PDB 6da0). Since NfGlck shares only 25% identity with the host enzyme (hGlck), we anticipate parasite specific inhibitors with anti-amoeba activity could be generated. To that end, we have screened a collection of known inhibitors of *Plasmodium falciparum* and T*rypanosoma brucei* hexokinases against NfGlck and have identified several small molecules with IC50 < 1µM that may serve as lead chemotypes for future therapeutic design. In efforts to genetically validate this target, we are working to develop molecular tools for use in *Naegleria* to assess the importance of potential target genes (such as NfGlck). Initially, a transfection approach will be optimized for the amoeba by modifying approaches used in other parasite systems. Technologies such as RNA interference and CRISPR/Cas9-mediated gene editing will be explored to determine the most efficient methods to manipulate *N.fowleri* genetically. Development of these techniques will provide an important tool to uncover potential genes that can be targeted for drug discovery.

P41. EuPathDB.org: Free, Online Resources Bringing Omics to Every Parasitologist

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The Eukaryotic Pathogen Database Resources (EuPathDB, http://eupathdb.org) are a family of 12 taxon-specific, free, online genome and other Omics data mining resources that support over 190 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. These resources facilitate the discovery of meaningful biological relationships (hypothesis testing) from large volumes of pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. The intuitive graphic interface allows users to take full advantage of the data without the need for programming. Data types range from genome sequence and annotation to transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using standard bioinformatics workflows and in-house analyses generate data including domain predictions and orthology profiles across all genomes, which permit inferences from data-rich organisms to organisms with limited or missing data. EuPathDB offers several perspectives for data mining - record pages compile all data for genes, pathways, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system that queries pre-analyzed data and returns genes or features with shared biological characteristics; a private Galaxy workspace for analyzing and viewing user data in context with public data already integrated into EuPathDB. These free resources easily merge evidence from diverse data and across species to place the power of bioinformatics with every scientist. Our active user support offers an email help desk, social media, video tutorials and a worldwide program of workshops. Please stop by our booth in the exhibit hall, to suggest a data set or see our newest addition, ClinEpiDB (http://clinepidb.org) which facilitates the exploration and analysis of epidemiologic studies.

P42. *Wolbachia* Whole-genome Sequencing in Five Different Isolates of *Dirofilaria immitis* with Different Macrocyclic Lactone Susceptibility Status

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Dirofilaria immitis, the pathogenic parasite responsible for heartworm disease, results in cardiopulmonary damage and severe complications in companion animals worldwide. Macrocyclic lactones (MLs) are the only drug approved by the Food and Drug Administration to prevent heartworm infection in dogs and cats. Since 2005, there have been 'lack of efficacy' reports, and ML-resistant isolates have been positively identified. *Wolbachia* is an endosymbiont of *D. immitis*. It is necessary for the host's reproduction, development, and survival, and plays an important role for pathogenesis in heartworm disease. It is therefore of interest to compare *Wolbachia* genomes from isolates of *D. immitis* with different ML susceptibility status. With the use of the immunomagnetic separation method and whole genome amplification, we prepared *Wolbachia* samples from two ML susceptible and three resistant *D. immitis* isolates, and then obtained genomic data through Illumina next-generation sequencing. *Wolbachia* sequences were also retrieved from the *D. immitis* genomic data in our laboratory. The mean depth of coverage of *Wolbachia* is approximately 40 - 75x, with more than 3,000 sequence variations found in each isolate. We identified 67 loci specific to resistant isolates but not susceptible isolates, including 22 genes affected. Phylogenetic analysis using 564 orthologous genes confirms that *Wolbachia* from two susceptible *D. immitis* isolates are indeed genetically different from the *Wolbachia* from resistant isolates. These results reveal the relationship between *Wolbachia* from five *D. immitis* isolates and suggest that the specific genetic markers in *Wolbachia* may have the potential to differentiate susceptible and resistant heartworm isolates.

P43. Comparison of Different Surveillance Methods for Modeling Tick Dispersal and Pathogen Prevalence for Selected Tick-borne Pathogens in Georgia

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Surveillance for ticks and tick-borne pathogens relies upon effective tick detection methods. Ticks are important vectors for human and domestic animal pathogens and active surveillance through tick collection is an effective way to assess risk to tick-borne disease. This study compares the tick abundance, species composition, and resource investment of two questing-tick collection methods (dragging and dry ice-baited dragging) and on-host wildlife tick collections. To account for known and suspected spatial and temporal influences on tick dispersal, each collection method was employed in two physiographic regions in Georgia (Piedmont and Lower Coastal Plain) and in three habitat types per region, with three replicate sites sampled for each habitat type (18 sites total). Each site was sampled seasonally (Fall 2017-Summer 2018) to account for known seasonal differences in tick abundance and species composition. Various wildlife groups were sampled with emphasis on passerine birds, rodents, and mesomammals. In total, 568 ticks of four species (*Amblyomma americanum* (91.73%), *Ixodes scapularis* (7.04%), I. affinis (0.70%), and *A. maculatum* (0.53%)) were collected via traditional tick drags, 1026 ticks of three species (*A. americanum* (95.13%), *I. scapularis* (4.68%), and *Dermacentor variabilis* (0.19%)) via dry ice-baited tick drags, and 420 ticks of 8 species (*D. variabilis* (28.57%), *I. scapularis* (25.71%), *A. americanum* (25.00%), *A. maculatum* (14.52%), *I. texanus* (5.00%), *Haemaphysalis leporispalustris* (0.71%), *I. muris* (0.24%), and *I. brunneus* (0.24%)) off wildlife hosts. Subsets of collected tick populations will be tested for various tick-borne pathogens including *Borrelia* spp., *Rickettsia* spp., *Ehrlichia ewingii*, *Ehlrichia chaffeensi*s, Panola Mountain *Ehrlichia*, and *Anaplasma phagocytophilum*. Comparing these active tick surveillance methods will provide important information for tick researchers and health officials to help them employ effective collection methods given their interests and resource limitations.

P44. Essential Mitochondrial Maintenance Proteins in the Parasite *Crithidia fasciculata*

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Crithidia fasciculata is a protozoan parasite that is non-pathogenic to humans, but is evolutionarily related to other trypanosomatids responsible for human illnesses affecting millions worldwide. Exclusive mosquito infectivity, low lab maintenance cost, and fast growth rate, makes *C. fasciculata* an ideal model organism to study trypanosomatids. The mitochondrion for unicellular organisms is comprised of various essential proteins that regulate cellular homeostasis and cell viability. Investigating functions of conserved mitochondrial proteins can lead to understanding of Trypanosomatid-specific pathways. The function and maintenance of the mitochondrial membrane may be aided by six uncharacterized proteins found in the *C. fasciculata* genome. The purpose of this study is to confirm mitochondrial localization for each of these proteins and investigate their contributions to changes in the mitochondrial membrane. Localization of all open reading frames was predicted to localize to the mitochondria. Mitochondrial proteins have a crucial role in trypanosomatid survival and infectivity. Disruption of these essential proteins may cause cellular apoptosis in these parasites. Understanding apoptosis in *C. fasciculata* is important for selecting drug targets in trypanosomatids that cause human diseases.

P45. Combined Genetic and Chemical Approaches Uncover the Essential Redox Network of the Human Malaria Parasite *Plasmodium falciparu*m

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Malaria remains a major health burden across the globe, with the most severe form of the disease caused by the eukaryotic parasite *Plasmodium falciparum*. Inside of the host red blood cell (RBC), *P. falciparum* maintains a complicated secretory pathway that originates in the endoplasmic reticulum (ER) and traffics proteins not only to the parasite plasma membrane, but also into the host cell, the vacuole in which the parasite lives, and into unique organelles required for the parasitic lifecycle. This trafficking network is predicated upon the folding of diverse, newly synthesized proteins within the ER, which requires the correct formation of intra- and intermolecular disulfide bonds (oxidative folding). In other eukaryotes, the Protein Disulfide Isomerase (PDI) family of enzymes has been identified as mediators of oxidative folding in the ER, but little has been done to characterize this process or these enzymes in *P. falciparum*. As a putative ER-resident chaperone with a thioredoxin domain, PfJ2 is a potential member of the *P. falciparum* PDI family. Using CRISPR/Cas9 editing of the parasite genome, we have confirmed localization of PfJ2 to the ER and shown through conditional knockdown that the protein is required for parasite survival in the RBC. Treatment of parasites with a crosslinker specific for redox-active cysteines traps clients to the PfJ2 thioredoxin domain. Identified potential substrates for this domain include other PDI family members and proteins localized throughout the secretory pathway of *P. falciparum*. The oxidative folding that occurs within the ER is essential for the parasite lifecycle, and therefore may be exploitable in the efforts to combat malaria, but the proteins that contribute to this process remain largely unexplored. Using a combination of CRISPR/Cas9 genetic editing and a redox-active crosslinker, we have begun to tease apart the pathway that leads to correct oxidative folding in the malaria parasite ER.

P46. Glutamate-gated Chloride Channels, Ivermectin and Pharyngeal Pumping in *C. elegans*

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Ivermectin targets *C. elegans* glutamate-gated chloride channels (GluCls) and results in a flaccid paralysis. Electropharyngeograms (EPG) record electrical signals emitted by pharyngeal muscles and neurons during pharyngeal pumping, the process by which the worm feeds. The aim of this technique is to record anthelmintic activity (effect of concentration and duration of treatment) of drugs targeting pharyngeal and extra-pharyngeal ion channels and neurotransmitters.Wild-type and mutant strains, with defects in various GluCl genes, of *C. elegans* (N2, Xa7400, JD608, JD740, JD105, DA1384 and CX12709) were treated with serotonin (10 µM) to stimulate pumping and subjected to increasing concentrations of ivermectin (None, 0.1, 0.3, 1, 3 and 10 nM). After 1h incubation, an EPG was performed for each ivermectin concentration (20 worms / concentration / 3 biological replicates) using the Screenchip Nemametrix system. The *glc-3* mutant (Xa7400), along with the *avr-15/avr-14/glc-1* triple mutant (JD608), the *avr-15/avr-14* double (JD740) and *avr-15* (JD105) single mutants were resistant to increasing IVM concentrations. When comparing the mutants and the N2 control in the absence of ivermectin, we observed identical pharyngeal pumping patterns in the JD608 and DA1383 strains. The other mutant strains showed a different pumping pattern. The *avr-15* and *avr-14* mutants showed less variability in their pumping frequency as well as shorter pump duration than the N2 control. The *glc-1* mutation seems to reverse the effects of the *avr-15* and *avr-14* mutations on pharyngeal pumping in the absence of ivermectin, indicating a potential action of the nervous system regulating pharyngeal pumping in *C. elegans*. We are continuing to measure the pumping properties of additional strains worm with further mutations in the GluCl to try and build a comprehensive model of the role of these channels in controlling this vital nematode behavior.

P47. Editing of Mucin-associated Surface Proteins (MASP) by CRISPR-Cas9 in *Trypanosoma cruzi*

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Trypanosoma cruzi is the causal agent of Chagas disease, which affects millions of people around the world, mostly in Latin America. *T. cruzi* is relatively unique in simultaneously expressing in its various life cycle stages, multiple variants of cell surface molecules encoded in large, multigene families, including trans-sialidases, mucins and mucin-associated surface proteins (MASP). Members of these families are likely generated by gene duplication and are also undergoing recombination to generate constant diversity. The MASP gene family is unique to *T. cruzi*, with no orthologues in other trypanosomatids. To date, the biological function for MASPs has not been determined, nor why *T. cruzi* has over 700 genes encoding variants of these surface proteins, although evasion of host immune responses is considered a likely reason. In this work, we attempted to truncate over 90% of the predicted MASP genes by targeting the conserved N-terminal secretion signal sequence with specific ribonucleoprotein complexes of CjCas9-gRNA and a DNA template with different peptide tags and stop codons. Homology-directed repair was confirmed by PCR and sequencing in the edited population. By using a split beta-galactosidase system, employing a beta-galactosidase alpha peptide in the repair template in an omega-fragment expressing *T. cruzi* strain, galactosidase activity confirmed the presence of the truncated MASP signal sequences in the parasite cytoplasm. This activity accumulated with repeated transfections, indicating increasing integration at the expected MASP loci. Soon after CRISPR editing, the tag+ population exhibited a slow growth rate, altered cell size and flagellum detachment and beta-gal+ parasites parasites were no longer detected at day 8 post-transfection, strongly suggesting death of the MASP gene-edited parasites. Additionally, surviving clones show only 1-4 edited genes and exhibit normal growth rate and morphology. These studies show that large-scale truncation of MASP proteins has a direct or indirect impact on *T. cruzi* epimastote survival.

P48. High Diversity of Cryptic Piroplasm Infections in Raccoons from Selected Areas of the United States and Canada

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The order Piroplasmida contains a diverse group of intracellular parasites, many of which can cause significant disease in humans, domestic animals, and wildlife. Two piroplasm species have been reported from raccoons (*Procyon lotor*), *Babesia lotori* (*Babesia* sensu stricto clade) and a species related to *Babesia microti* (called *B.microti*-like sp.). The goal of this study was to investigate diversity, distribution, and prevalence of *Babesia* in raccoons. We tested raccoons from selected regions in the United States and Canada for the presence of *Babesia* sensu stricto and *Babesia microti*-like sp. piroplasms. Infections of *Babesia microti*-like sp. were found in nearly all locations sampled, while *Babesia* sensu stricto infections were more common in the Southeastern United States (20-45% prevalence). Co-infections with both *Babesia* sp. were common. Sequencing of the partial 18S rRNA and cytochrome oxidase subunit 1 (*cox1*) genes led to the discovery of two new *Babesia* species, both found in several locations in the eastern and western United States. One novel *Babesia* sensu stricto sp. was most similar to *Babesia gibsoni* while the other *Babesia* species was present in the 'western piroplasm' group and was related to *Babesia conradae*. Phylogenetic analysis of the cox1 sequences indicated possible eastern and western genetic variants of these species as well as the *B. lotori* clade. Additional analyses are needed to characterize these novel species; however, this study indicates there are now at least four species of piroplasms infecting raccoons in the United States and Canada (*Babesia microti*-like sp., *Babesia lotori*, a novel *Babesia* sensu stricto sp., a novel western *Babesia* sp.) and a possible fifth species (*Babesia* sensu stricto) in raccoons in Japan.