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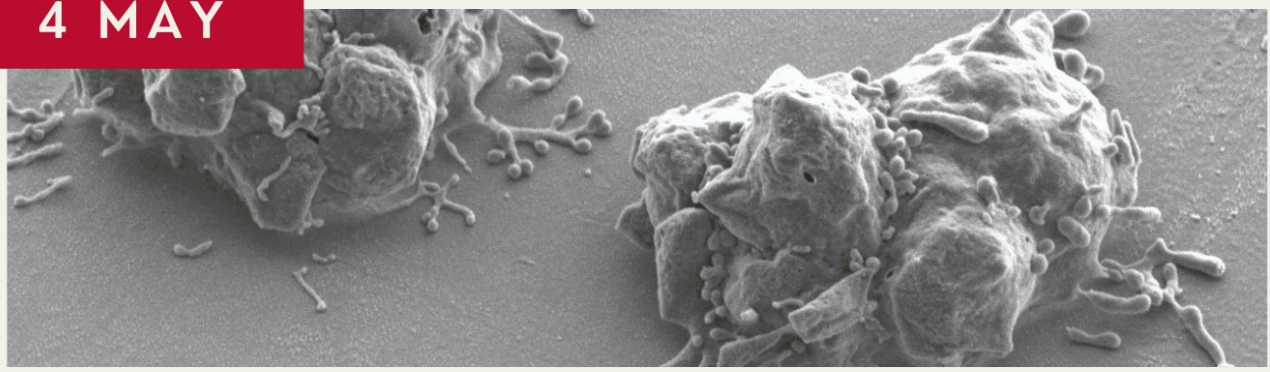
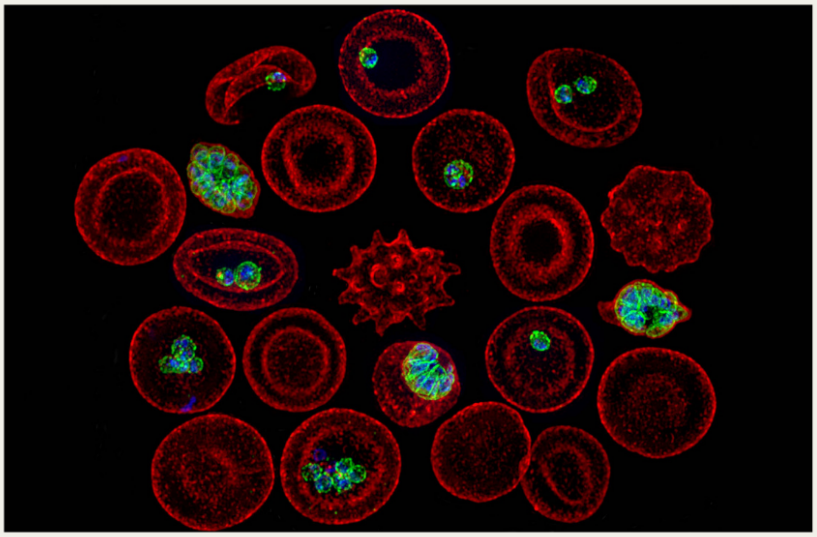
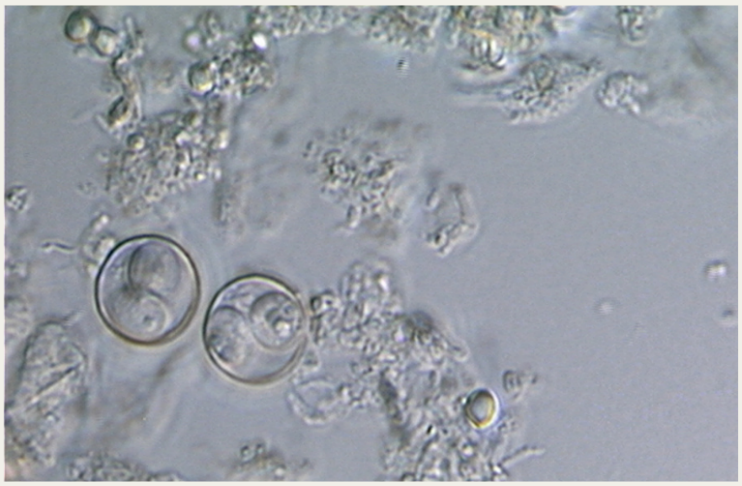




Georgia Center for Continuing Education

31st Annual

MOLECULAR PARASITOLOGY & VECTOR BIOLOGY SYMPOSIUM



ATHENS, GEORGIA

Abstract Book

2022

Center for Tropical & Emerging Global Diseases
UNIVERSITY OF GEORGIA

Global Health Through Research

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Program

8:15 AM Registration and Poster Set-up

9:00 AM Opening Remarks: Dennis Kyle, Director of CTEGD

SESSION 1 — Moderators: Abigail Calixto, Justine Shiau, Anna Gioseffi

9:10 AM **Melissa Sleda**, CETGD and Dept. of Cellular Biology, UGA
The mitochondrial ubiquinone synthesis is a new drug target in both acute and chronic stages of *Toxoplasma gondii*

9:30 AM **Emily Knight**, Dept. of Genetics & Biochemistry, Clemson University
A Novel peroxin is required for mitochondrial morphology: Implications for resolving a new peroxisome-mitochondrial contact site

9:50 AM **Megan Beaudry**, CTEGD and Dept. of Environmental Health Science, UGA
A new targeted library enrichment applied to human infecting *Cryptosporidium* ssp. for whole genome sequencing

10:10 AM **BREAK — POSTER VIEWING (even posters)**

SESSION 2 — Moderators: Justine Shiau, Ruby Harrison, Benjamin Phipps

10:50 AM **Camila Marques da Silva**, CTEGD and Dept. of Cellular Biology, UGA
Type 1 interferons drive cell-autonomous defenses within *Plasmodium*-infected hepatocytes

11:10 AM **INTRODUCTION OF EARLY CAREER SCHOLAR - Belen Cassera**

11:15 AM **Filipa Rijo-Ferreira**, University of California, Berkeley School of Public Health
Circadian rhythms in parasitic diseases

12:10 PM **LUNCH — POSTER VIEWING (12:40 Trivia in Mahler Hall)**

SESSION 3 — Moderators: Melissa Sleda, Abigail Calixto, Megna Tiwari

1:10 PM **Gina Marcela Gallego-Lopez**, Morgridge Institute for Research and Dept. of Medical Microbiology & Immunology, University of Wisconsin-Madison
Kiss and spit metabolomics highlights the role of the host cN-II enzyme on purine metabolism during *Toxoplasma gondii* infection

1:30 PM **Rudo Kieft**, Dept. of Biochemistry & Molecular Biology, UGA
PP1 regulates transcription termination in *Leishmania major*

1:50 PM **Benjamin Phipps**, CTEGD and Dept. of Genetics, UGA
Ecdysteroids synthesized post-blood meal regulate egg formation in the Indian malaria vector *Anopheles stephensi*

1:50 PM **David Anaguano**, CTEGD and Dept. of Cellular Biology, UGA
Rhoptry Neck Protein 11 plays an essential role in *P. falciparum* invasion of erythrocytes

2:30 PM **BREAK — POSTER VIEWING (odd posters)**

SESSION 4 — Moderators: Benjamin Phipps, Megna Tiwari, Melissa Sleda

3:10 PM **Sabrina Elizabeth Cline**, CTEGD and Dept. of Cellular Biology, UGA
Elucidating the role of inositol-tetrakisphosphate 1-kinase in *Trypanosoma cruzi*

3:30 PM **A. Cassiopeia Russell**, CTEGD and Dept. of Infectious Diseases, UGA
Characterization of the extracellular vesicles secreted by *Naegleria fowleri*

3:50 PM **INTRODUCTION OF THE KEYNOTE SPEAKER - Vasant Muralidharan**

4:00 PM **Philippe Bastin**, Trypansome Cell Biology Unit, Institut Pasteur
Single cell RNA sequencing reveals trypanosome development in the salivary glands of the tsetse fly

Poster Presentations

P1 **Nicole Khamsa,** The University of Georgia
Establishing a reporter system to identify membrane contact site components between the apicoplast and mitochondrion of *Toxoplasma gondii*

P2 **Justine Shiau**, The University of Georgia
In vitro *Plasmodium falciparum* liver-stage biology

P3 **Ashley Dombrowski**, Dept. of Entomology, UGA
Effects of coprophagy on bacterial acquisition and competition in triatomine kissing bugs

P4 **Victoria Mendiola**, The University of Georgia
Visualization and quantification of Artemisinin-induced dormant and dead *Plasmodium falciparum*

P5 **Andrés Tibabuzo Perdomo**, Dept. of Medical Microbiology & Immunology, University of Wisconsin - Madison
Hide and seek: The importance of lipoxygenases in *Toxoplasma gondii* for immune evasion

P6 **Aylla von Ermland**, Dept. of Cellular Biology, UGA
Modification of dsDNA break repair mechanisms in *Trypanosoma cruzi*

P7 **Alejandra Villegas Lopez**, The University of Georgia
Breaking out: Egress of malaria parasites requires a putative glycosyltransferase

P8 **Edward D'Antonio**, University of South Carolina Beaufort
The role of Phe-337 in *Trypanosoma cruzi* glucokinase: Thermodynamic evaluation on the binding interaction of glucosamine-based inhibitors

P9 **Nupur Kittur**, Center for Tropical & Emerging Global Diseases, UGA
ClinEpiDB.org: An open access platform for sharing and exploring global health datasets

P10 **Emily Bremers**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Elucidating the mechanism of resistance of b-carboline derivatives

P11 **Reagan Haney** , CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Identification of beta-carboline derivatives active against quiescent artemisinin-resistant *Plasmodium falciparum* parasites

P12 **Watcharatip Dedkhad**, Center for Tropical & Emerging Global Diseases, UGA
The regulation of proteolytic cascade in egress of *Plasmodium falciparum*

P13 **Lasya R. Penumarthi**, Institute of Bioinformatics, UGA
Comparative analyses of a newly sequenced and annotated *C. meleagridis* genome

P14 **Susanne Warrenfeltz**, The University of Georgia
VEuPathDB: Omics support for the global parasite, vector and fungal research communities

P15 **Madelaine Usey**, CTEGD and Dept. of Cellular Biology, UGA
Characterizing the *T. gondii* homolog of ATPase Inhibitory Factor 1 (IF1)

P16 **Grace Woods**, The University of Georgia
Function of conserved transmembrane proteins in *Plasmodium* egress

P17 **Ash Pathak**, CTEGD and Dept. of Infectious Diseases, UGA
Malaria@TheSporoCore, where mosquitoes are more than just a flying syringe

P18 **Natalie Wilson**, Dept. of Infectious Diseases, UGA
Identification of new genes and pathways contributing to ivermectin hypersensitivity and resistance in *C. elegans* based on transcriptomics data from *B. malayi*

P19 **Carissa Gilliland**, Dept. of Entomology, UGA
Roles of the microbiome in immune system function in kissing bugs

P20 **Rui Xiao**, Institute of Bioinformatics, UGA
Single-molecule full-length Iso-seq data reveal and help explain *Cryptosporidium parvum*'s transcriptional landscape

P21 **Clayton Parker**, The University of Georgia
Analysis of the PJW/PP1 complex involved in RNA Pol II transcription termination in trypanosomes

P22 **Nia I. Keyes-Scott**, The University of Georgia
Understanding the role of bacterial symbiont *R. rhodnii* in kissing bug lipid metabolic physiology

P23 **Mayara S. Bertolini**, CTEGD and Dept. of Cellular Biology, UGA
Phosphoinositide phospholipase C is essential for the infective stages of *Trypanosoma cruzi* but is not involved in the synthesis of inositol pyrophosphates

P24 **Katherine Moen**, CTEGD and Dept. of Cellular Biology, UGA
Protein disulfide isomerase of *Toxoplasma gondii*

P25 **Baihetiya Baierna**, CTEGD and Dept. of Cellular Biology, UGA
Characterization of two methyltransferases in *Toxoplasma gondii* ubiquinone biosynthesis pathway

P26 **James Oristian**, Center for Tropical & Emerging Global Diseases, UGA
Induced in vitro sexual commitment of *Plasmodium cynomolgi*

P27 **Ọla Fagbami**, Center for Tropical & Emerging Global Diseases, UGA
Sweet danger: Protein glycosylation in human malaria parasites

P28 **Essel Charles-Chess**, CTEGD and Dept. of Cellular Biology, UGA
Memory regulatory T cells offer protection from malaria reinfection

P29 **Amrita Sharma**, Dept. of Molecular & Cellular Biology, Kennesaw State University
Evaluation of carbazole derivatives as leads for human African trypanosomiasis drug development

P30 **Msano Mandalasi**, CTEGD, Dept. of Biochemistry & Molecular Biology, and CCRC, UGA
Oxygen-dependent regulation of F-box proteins in *Toxoplasma gondii*

P31 **Abigail Calixto**, CTEGD and Dept. of Cellular Biology, UGA
A *Toxoplasma gondii* calcium/proton exchanger and its role in regulating acidic Ca^{2+} stores and Ca^{2+} uptake by the endoplasmic reticulum

P32 **Anthony A Ruberto**, Center for Tropical & Emerging Global Diseases, UGA
Single-cell RNA profiling of *Plasmodium vivax*-infected hepatocytes reveals parasite- and host-specific transcriptomic signatures and therapeutic targets

P33 **Miryam Andrea Hortua**, Center for Tropical & Emerging Global Diseases, UGA
The role of the phosphatidylinositol phospholipase C in the synthesis of inositol polyphosphates of *Toxoplasma gondii*

P34 **Molly Bunkofske**, The University of Georgia
Epitopes in the GPI attachment signal peptide of *Trypanosoma cruzi* mucin proteins generate robust but delayed and nonprotective CD8+ T cell responses

The mitochondrial ubiquinone synthesis is a new drug target in both acute and chronic stages of *Toxoplasma gondii*

Melissa A. Sleda, Zhu-Hong Li, Baihetiya Baierna, Catherine Li, Silvia NJ Moreno
Dept. of Cellular Biology, University of Georgia, Athens, GA 30602

The current treatments against toxoplasmosis are ineffective because they only inhibit the actively growing tachyzoite stage while having little effect on the slow growing bradyzoite form, commonly found in tissue cysts. The mitochondrion of *T. gondii* is essential for its survival and is a validated drug target. Several antiparasitic drugs, such as atovaquone and endochin-like quinolones, act through inhibition of the mitochondrial electron transport chain at the coenzyme Q:cytochrome c oxidoreductase. The coenzyme Q or ubiquinone (UQ) molecule consists of a water soluble quinone head and a lipophilic isoprenoid tail that anchors UQ to membranes. The synthesis of UQ has not been studied in *T. gondii*, although the synthesis of the isoprenoid unit is known to be essential for cell growth. Previously we characterized the enzyme (TgCoq1) responsible for the synthesis of the isoprenoid chain that supplies the tail of the UQ and found it to be essential for parasite growth. We also discovered a lipophilic bisphosphonate, BPH-1218, that specifically inhibits TgCoq1 and protected mice against a lethal infection with *T. gondii*. In this work we developed a rescue screen protocol using supplementation of UQs which led to the discovery of new inhibitors of the UQ pathway. Two compounds inhibited replication of Me49 (a type II cyst forming strain) and reduced the size of in vitro bradyzoite vacuoles. These compounds also reduced the number, and size of plaques, of viable ex vivo derived and in vitro differentiated bradyzoites. Most interestingly, BPH-1218 and NV-32, another bisphosphonate, was able to reduce the number of tissue cysts in the brains of chronically infected mice.

A novel peroxin is required for mitochondrial morphology: Implications for resolving a new peroxisome-mitochondrial contact site

Emily Knight, Logan Crowe, Andrew Gianos, Meredith Morris
Dept. of Genetics and Biochemistry, Clemson University, Clemson, SC

Peroxisomes are dynamic and ubiquitous organelles that house many metabolic pathways and interact with other organelles such as the endoplasmic reticulum, lipid droplets, and mitochondria. One mechanism for organelle interaction is through membrane contact sites. While contact sites between multiple organelles have been identified, little is known about the proteins that serve as molecular tethers in such sites. We study organelle dynamics using peroxisome-like organelles called glycosomes in the early diverging organism *Trypanosoma brucei* and have identified a novel peroxin (protein involved in peroxisome biogenesis) that is essential for mitochondrial morphology. Silencing this protein leads to a significant growth defect and swollen mitochondria. Multiple mitochondrial membrane transport channels have been identified in immunoprecipitation studies. Based on these findings, we hypothesize that this protein that we have named a putative peroxisome involved in mitochondrial morphology (PIMM), localizes to glycosomes and mitochondria at contact points, which facilitate the transfer of metabolites between the two organelles. Disruption of this connection results in “leaky” mitochondria and cell death. Current work is focused on resolving the metabolic defects in PIMM-deficient cells and identifying additional molecular components of these contact sites. This work forwards our understanding of how contact sites are established and the role they play in interorganelle communication.

A new targeted library enrichment applied to human infecting *Cryptosporidium* ssp. for whole genome sequencing

Megan S. Beaudry[1,2], Asis Khan[3], Natalia Bayona Vasquez[4,5], Rodrigo P. Baptista[1,2,#], Michael E. Grigg[6], Jessica C. Kissinger[1,6,7], and Travis C. Glenn[2,6,7]

[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA USA [2]Dept. of Environmental Health Science, University of Georgia, Athens, GA USA. [3]Animal Parasitic Diseases Laboratory Agricultural Research Service, United States Dept. of Agriculture, Beltsville, MD, USA [4]Division of Natural Science and Mathematics, Oxford College at Emory University, Oxford GA, USA. [5]Institute of Bioinformatics, University of Georgia, Athens, GA USA [6]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA [7]Dept. of Genetics, University of Georgia, Athens, GA USA [#]Present address: Houston Methodist Research Institute, Houston, TX, USA

Cryptosporidium spp. are zoonotic protozoan parasites that cause severe illness in vulnerable populations, including the largest waterborne-outbreak in US history. However, work with clinical and environmental samples containing *Cryptosporidium* spp. is challenging, as there is limited target DNA, few molecular genetic tools and there are no animal models to culture some species. Here, we develop a set of hybridization capture baits (i.e., WGS Crypto-Cap) to enrich DNA for the whole genomes of a cluster of six human infecting species of *Cryptosporidium* that are highly genetically similar to each other (i.e., *C. cuniculus*, *C. hominis*, *C. meleagridis*, *C. parvum*, *C. tyzzeri*, and *C. viatorum*). The core of the bait set is designed from the *C. parvum* genome, from which unique genomic regions were pulled out from each of the aforementioned species. We demonstrate the efficiency of our bait set on several species of *Cryptosporidium* both in silico and in vitro. In silico simulations reveal that WGS Crypto-Cap has the potential to achieve 67-97.9% coverage for all species in the bait set. In vitro testing on pure samples demonstrates the ability of the baits to cover 99% of the *C. parvum* genome. Sensitivity testing on pure samples (i.e., *C. parvum*) diluted in a complex DNA background community shows the ability of the assay to efficiently capture the genome of samples containing 0.1 ng of target material in a complex background. In host-associated clinical samples, our bait set increases the proportion of on-target reads up to 2000-fold, and can provide greater coverage and depth than unenriched libraries. Additionally, our bait set accurately teases apart simulated mixed infection samples in vitro. The development of the WGS Crypto-Cap bait set will assist in key areas for public health, by allowing researchers to accurately and efficiently sequence the whole genome of multiple species of *Cryptosporidium*.

Type 1 interferons drive cell-autonomous defenses within *Plasmodium*-infected hepatocytes

Camila Marques-da-Silva[1] and Samarchith P. Kurup[1]

Center for Tropical and Emerging Global Diseases, Dept. of Cellular Biology, University of Georgia, Athens, GA, USA

Malaria, caused by *Plasmodium* parasites is a global health emergency. The sporozoite stage of *Plasmodium* inoculated into the host by mosquitoes initiate malaria by migrating to the liver and establishing an infection in the hepatocytes. Although natural immune responses mediated by type I interferons are pivotal in controlling such infections, how they do so has remained unknown. Analysis of the transcriptional profiles of primary murine hepatocytes infected with *Plasmodium berghei* (Pb)- indicated a type I interferon (IFN) signaling signature in such cells. A key immune pathway enriched in Pb infected hepatocytes was the oxidative stress response cascade. In agreement with this finding, we observed that type I IFNs induced reactive oxygen species (ROS) in Pb-infected hepatocytes, and the administration of anti-oxidants hindered the control of malaria in the liver. We show that ROS induced lipidation of LC3, resulting in the localizations of LC3, lysosomes, and Guanylate Binding Protein (GBP)-1 to the intracellular *Plasmodium*, potentially resulting in the lysis of the parasite within the hepatocyte. Corroborating these findings, blocking lysosomal function or deleting GBPs in mice resulted in suboptimal control of liver-stage malaria. Our results define a novel molecular pathway of natural control of liver-stage malaria.

Kiss and spit metabolomics highlights the role of the host cN-II enzyme on purine metabolism during *Toxoplasma gondii* infection

Gina M. Gallego-Lopez[1,2], William J. Olson[2], Andres M. Tibabuzo-Perdomo[2], David Stevenson[3], Daniel Amador[3] and Laura J. Knoll[2]

[1]Morgridge Institute for Research, Madison, WI, 53706, USA [2]Dept. of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, 53706, USA [3]Dept. of Bacteriology, University of Wisconsin-Madison, Madison, WI, 53706, USA

The obligate intracellular apicomplexan parasite *Toxoplasma gondii* is a public health threat, particularly to immunodeficient populations. *T. gondii* relies on the host cell to provide metabolites the parasite is incapable of synthesizing, including arginine, tryptophan, tyrosine, and purines. Despite the importance of host metabolism in the successful replication of the parasite, we have limited understanding of how *T. gondii* changes it. One mechanism for host cell manipulation is the pre-invasion process known as kiss and spit, when the contents of the parasite rhoptry organelles are secreted into the host cytoplasm. Our study used mass spectrometry-based metabolomics to determine how kiss and spit changes host metabolism. Kiss and spit altered metabolite abundance in nucleotide synthesis, the pentose phosphate pathway, glycolysis, and amino acid synthesis. An increase in 2,3-bisphosphoglycerate (2,3-BPG) abundance led us to hypothesize that high levels of host 2,3-BPG contribute to the activation of host cytosolic nucleosidase II (cN-II) and alters purine availability. Treatment with the cN-II inhibitor fludarabine and a cell line with a cN-II genetic knock-out halted *T. gondii* growth by affecting the availability of purines for the parasite. Taken together our results demonstrate that *T. gondii* kiss and spit remodels host metabolism and manipulates the host cN-II enzyme to acquire their purine metabolites.

PP1 regulates transcription termination in *Leishmania major*

Rudo Kieft[1], Haidong Yan[2], Robert J. Schmitz[2], and Robert Sabatini[1]

[1]Dept. of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia, 30602, USA [2]Dept. of Genetics, University of Georgia, Athens, Georgia, 30602, USA

The genomes of kinetoplastids are organized into polycistronic transcription units that are flanked by a novel modified DNA base (base J, b-D-glucosyl-hydroxymethyluracil). Previous work has established a role of base J in promoting RNA polymerase II termination in *Leishmania major* and *Trypanosoma brucei* where the loss of J leads to termination defects and expression of downstream genes. We recently identified a PJW/PP1 complex in *Leishmania* containing a J-binding protein (JBP3), PP1 phosphatase 1, PP1 interactive-regulatory protein (PNUTS) and Wdr82. Genetic and biochemical analysis of the complex in *T. brucei* suggested it regulates transcription termination by recruitment to termination sites via JBP3-base J interactions and dephosphorylation of specific proteins by PP1, similar to the control of termination in higher eukaryotes. However, the *T. brucei* complex lacked an obvious PP1 ortholog and thus we were unable to address the role of the sole catalytic component in Pol II transcription termination. We now demonstrate that deletion of the PP1 component of the PJW/PP1 complex in *L. major* leads to read through transcription at the 3'-end of polycistronic gene arrays and expression of downstream genes similar to defects in cells with reduced base J. These findings significantly expand our understanding of the mechanism of Pol II transcription termination in highly divergent organisms that utilize polycistronic transcription and need to decouple termination from 3'-end formation of individual genes.

Ecdysteroids synthesized post-blood meal regulate egg formation in the Indian malaria vector *Anopheles stephensi*

Benjamin L. Phipps[1,3], Mark R. Brown[2,3], Michael R. Strand[2,3]
[1]Dept. of Genetics, [2]Dept. of Entomology, [3]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602

Mosquitoes feed on blood to obtain nutrients required for egg production. In the dengue vector *Aedes aegypti*, blood-feeding triggers the release of insulin-like peptides (ILPs) and ovary ecdysteroidogenic hormone (OEH) from the brain. ILPs and OEH then stimulate the ovaries to synthesize ecdysteroids, which regulate production of the yolk protein vitellogenin (Vg) in the fat body and egg maturation. Less understood are the processes that regulate egg formation in anopheline mosquitoes, which transmit mammalian malaria parasites. Female anopheline mosquitoes acquire ecdysteroids from males by mating but also synthesize ecdysteroids after blood feeding. In contrast, it is unknown whether ecdysteroids acquired by males have roles in producing eggs or whether ILPs and OEH regulate ecdysteroid production in the ovaries. To address these questions, we assessed the effects of mating on ecdysteroid titers and fecundity in *Anopheles stephensi*. We detected no difference in ecdysteroid titers or Vg expression between mated and unmated females. Functional assays further identified ILP3, ILP4 and 20-hydroxyecdysone (20E) as the key factors that regulate egg formation in *An. stephensi*. Overall, our results indicate ecdysteroids produced by females after blood feeding, rather than the ecdysteroids acquired from males control yolk biosynthesis in *An. stephensi*. Our results further suggest ILPs are the key factors that activate ecdysteroid production in anopheline mosquitoes after blood feeding.

Rhoptry Neck Protein 11 plays an essential role in *P. falciparum* invasion of erythrocytes

David Anaguano-Pillajo[1,2], Vasant Muralidharan[1,2]
[1]Dept. of Cellular Biology, University of Georgia, Athens GA. [2]Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia, Athens, GA

Malaria is a global and deadly human disease caused by the apicomplexan parasites of the genus *Plasmodium*. Infection with one species, *Plasmodium falciparum*, causes most of the global mortality. Parasite proliferation within human red blood cells (RBC) is associated with the clinical manifestations of the disease. To adequately establish infection within the human host, a key aspect of *P. falciparum* life cycle is the invasion of RBCs, which is known to be mediated by the secretion of effectors from different secretory organelles from merozoite-stage parasites. One of these key organelles is the rhoptry, which undergoes dynamic physiological changes during invasion, harbors a group of effectors essential for this process. One of these effectors is the rhoptry-localized protein Rhoptry Neck Protein 11 (RON11), which contains 7 transmembrane domains and a putative calcium-binding EF-hand domain. Using the Tet-Apt inducible knockdown system, we have shown RON11 is essential for intraerythrocytic development and more specifically for merozoite invasion. Using immunofluorescence assays, we confirmed RON11 localization to the rhoptry neck, and its consequent migration to the apical end of the merozoites during their attachment to RBCs. Also, we have shown RON11 knockdown does not affect expression or secretion of rhoptry effectors during invasion. Our preliminary data shows that RON11 plays an essential role in merozoite invasion.

Elucidating the role of inositol-tetrakisphosphate 1-kinase in *Trypanosoma cruzi*

Sabrina Elizabeth Cline[1], Adolfo Saiardi[2], & Roberto Docampo[1]
[1]Center for Tropical and Emerging Global Diseases and Dept. of Cellular Biology, University of Georgia, GA 30602-7400, USA [2]Medical Research Council Laboratory for Cell Biology, University College London, WC1E 6BT London, United Kingdom

Trypanosoma cruzi is an intracellular parasite that is the causative agent for Chagas Disease, a chronic bloodborne infection endemic to the Americas that has infected eight million individuals and puts another eighty million at risk of infection. Ubiquitous to eukaryotic survival, inositol phosphates (IPs) are involved in a myriad of biological roles and activities such as calcium signaling, phosphate homeostasis, energy metabolism, and disease pathogenicity. In *Saccharomyces cerevisiae*, inositol polyphosphate (IPP) synthesis occurs through the phosphoinositide phospholipase C (PLC)-catalyzed hydrolysis of PIP2 into IP3 and diacylglycerol and further IP3 phosphorylation by additional kinases. Recently, inositol-tetrakisphosphate 1-kinase (ITPK1) has been described as an enzyme that may mediate a lipid-independent IP6 soluble synthesis pathway through phosphorylation of IP1 and other intermediates in the cytosol. Preliminary bioinformatics research identified hypothetical protein TcCLB.503885.50 as a *T. cruzi* ITPK1 (TcITPK1) homolog. Phylogenetic and structural bioinformatic studies have established TcITPK1 as orthologous to other high-order eukaryotic ITPK1s including Homo sapiens ITPK1, a protein previously established to follow the lipid-independent IPP synthesis pathway. The ability of TcITPK1 to act as the mediator for this alternative pathway has been established through plc1Δ yeast complementation assays and SAX-HPLC analysis of radioactively labeled inositol in complemented yeast. Immunofluorescence assays of *T. cruzi* epimastigotes with endogenously tagged TcITPK1 have shown this protein to localize to the cytosol, the site of the lipid-independent IPP synthesis pathway. Additionally, preliminary data demonstrates TcITPK1 may be essential to *Trypanosoma cruzi* survival as multiple CRISPR/Cas9 knockout experiments have resulted in no viable in vitro parasites.

Characterization of the extracellular vesicles secreted by *Naegleria fowleri*

A. Cassiopeia Russell[1,2], Dennis E. Kyle[1,2,3]
[1]Center for Tropical and Emerging Global Diseases, Athens, GA, USA. [2]Dept. of Infectious Diseases, University of Georgia, Athens, GA, USA. [3]Dept. of Cellular Biology, University of Georgia, Athens, GA, USA

The pathogenic free-living amoeba, *Naegleria fowleri* is the causative agent for primary amoebic meningoencephalitis, an acute brain disease with a case mortality rate of >97%. Several factors contribute to this rate of mortality including delayed diagnosis, ineffective therapeutics and lack of understanding of the amoebic pathogenesis. Recently, there have been advances in the study of the parasite biology, but the understanding of the molecular basis for parasite-host interactions is lacking. Apparatuses termed extracellular vesicles (EVs) have been implicated in the field of intercellular communication. These EVs are secreted from cells and house various signaling molecules that elicit a response in recipient cells. One class of molecules found in these vesicles are microRNAs, which are small secretory molecules that can be detected in many bodily fluids. These have been implicated as regulators of gene expression, can modulate host immune responses, and are used as biomarkers for the diagnosis and prognosis of various diseases. Thus we reasoned that *N. fowleri* might also produce microRNAs and proteins and package them into EVs to be secreted for similar purposes. We show that EVs are secreted by *N. fowleri* and Nanoparticle Tracking Analysis details particle enumeration and size determination. We confirmed the size of these EVs and determined possible routes of secretion by scanning electron microscopy. Deep-sequencing of the RNA contents of these EVs followed by computational processing has uncovered a highly prevalent smallRNA that we have validated with qPCR in EV RNA from several *N. fowleri* clinical isolates and in infected mouse serum. Mass spectrometry has identified thousands of proteins within the secreted vesicles, and R18 fusion assays show that these EVs are taken up by host-cells and other amoebae. This and future work will expand the knowledge of intracellular interactions among these amoebae and potentially provide an additional option for diagnosis of this infection.

Poster Presentations

P1. Establishing a reporter system to identify membrane contact site components between the apicoplast and mitochondrion of *Toxoplasma gondii*

Nicole Khamsa
The University of Georgia

In eukaryotes, membrane contact sites (MCS) are defined as specialized regions between two organelles in close apposition to each other. These regions enable inter-organellar communication, like the exchange of metabolites, by creating microdomains maintained via protein complexes. *Toxoplasma gondii* is an obligate intracellular protozoan parasite and the causative agent of disseminated toxoplasmosis, which is clinically relevant for mothers during pregnancy and immunocompromised individuals. Due to their complex life cycle, *T. gondii* must adapt its metabolic pathways to its ever-changing environment, which requires inter-organellar communication. *T. gondii* harbors two essential organelles: the apicoplast, a non-photosynthetic plastid conserved in most apicomplexans, and the mitochondrion. The two organelles have been observed in close proximity for many years and share essential metabolic pathways including the isoprenoid and heme biosynthesis pathways. Despite their close apposition and their metabolic exchanges, little is known about the nature of their interaction. We hypothesize that apicomplexan-specific MCS proteins mediate the interaction between the apicoplast and the mitochondrion of *T. gondii*. To identify those interactors, we are creating a reporter system using SplitGFP which consists of two halves of a green fluorescent protein that do not fluoresce on their own but can self-complement and fluoresce when they interact. This system can be used as a fluorescent tag to study protein-protein interactions in live cells. As a proof of concept, we are generating strains with each component of the SplitGFP system in different subcellular compartments of *T. gondii*. Once established, we will use this split system as a reporter system in a genetic screen to identify MCS components between the apicoplast and mitochondrion of *T. gondii*. Understanding apicomplexan-specific MCS will shed light on inter-organelle communication in these organisms and on essential proteins that may be targeted in the on-going search to find new therapeutic drug targets against *T. gondii* infections.

P2. In vitro *Plasmodium falciparum* liver-stage biology

Justine C. Shiau, Anne Elliot, Dennis E. Kyle
University of Georgia

Recent advances in establishing *Plasmodium falciparum* liver stages in human hepatocytes have provided new insights into human hepatocyte host-parasite interactions. Amongst the liver-stage tools, the humanized mouse model has been used extensively in recent years for genetic crosses, while the in vitro primary human hepatocyte (PHH) model allows high-resolution imaging and quantification of the liver-stage infection. Here we present our work-in-progress of the in vitro liver stage culture characterization and optimization to ultimately achieve parasite's transition to blood-stage infections. We performed and characterized infections of field-derived *P. falciparum* isolates with PHH donors to select a competent host and parasites pair and optimize culture gas conditions to promote parasite growth. We have identified competent hepatocyte donors and parasite strains for further optimizations. Moreover, we found low oxygen culture conditions enhance schizont development during the first week of the liver-stage infection. Although further optimization is required for the liver-to-blood stage transition, the current model can provide insights on the liver-stage biology and have the potential to further our understanding of host-parasite interactions during the quiescent stage of malaria infection. Lastly, the utilization of field isolates may also provide a glimpse of the ever-changing transmission dynamic out in the field.

P3. Effects of coprophagy on bacterial acquisition and competition in triatomine kissing bugs

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Triatomine kissing bugs, including *Rhodnius prolixus*, are the primary vectors of *Trypanosoma cruzi*, the causative agent of Chagas Disease. These obligately and exclusively blood-feeding insects must take repeated blood meals to successfully develop into adults and reproduce. Other exclusive blood-feeding insects such as bed bugs and Tsetse flies maintain intracellular, endosymbiotic bacteria that provide their host with B vitamins– essential nutrients that are not abundant in vertebrate blood. In contrast, *R. prolixus*' symbionts exist in the gut and are acquired through coprophagy, the process by which insects ingest infected excrement. Kissing bugs have a limited diversity of bacteria present in their gut but little is known about how these non-symbiotic bacteria are acquired. This project's objective is to ascertain whether various bacterial species can be acquired through coprophagy. To execute this project, the bacterial presence in fecal matter was quantified through serial dilutions and plating. Axenic nymphs (raised from sterilized eggs) were exposed to cardstock saturated in the fecal matter of bugs fed a singular bacterial species, then bugs were fed a sterile blood meal. Following this coprophagic activity, the exposed bugs underwent DNA extractions and quantitative PCR (qPCR) with gene-specific primers to test for the presence of bacteria after a bloodmeal. We then tested if concentration of fecal matter influenced bacterial titer in the insect. Our results suggest that not only can these bugs pick up bacteria through coprophagy, but some bacteria are more successful at colonizing kissing bugs than others. We also investigated if the time of colonization of bacteria influences bacterial titer and microbiome composition. Further understanding the dynamics that shape the kissing bug microbiome will increase our knowledge of kissing bug physiology.

P4. Visualization and quantification of Artemisinin-induced dormant and dead *Plasmodium falciparum*

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Artemisinin (ART) drugs are the most important drugs used to treat malaria due to the rapid onset of action and broad-stage activity against symptom causing erythrocytic stages of the *Plasmodium* spp. parasite. However, the emergence of ART-resistant parasites challenges ART combination therapies, research, and diagnostic methods. In addition to resistance, ART and its derivatives induce dormancy in ring-stage parasites leading to an unknown proportion of these parasites that can recover and cause recrudescent disease. Difficulties lie in the inability to differentiate dormant versus dead parasites due to the similar morphology in Giemsa-stained blood smears. We aimed to explore new methods to differentiate dead versus dormant ring-stage parasites and to quantify the proportion that recover from dormancy. We have assessed nuclear, cytoplasmic, and mitochondrial stains for use in long term, live-cell imaging. Importantly, we found that many commonly used stains are toxic and prevent dormant parasites from recovering. Instead, we have developed new protocols for imaging at extended time points to capture not only the morphology of dead and dormant parasites, but to quantify the proportion of parasites that recover. These methods are now being used to assess recovery rates for ART-susceptible and -resistant *P. falciparum* clones.

P5. Hide and seek: The importance of lipoxygenases in *Toxoplasma gondii* for immune evasion

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Toxoplasma gondii is a successful parasite and current estimates show that at least 1/3rd of the population has been infected. The most vulnerable populations affected by *T. gondii* are immunocompromised people and pregnant women. One defining characteristic of this parasite is that it only undergoes sexual development in the gut of felines, its definitive host. Recently, our lab discovered that felines lack a key enzyme that leads to the accumulation of linoleic acid in the small intestine of felines. This abundance, in turn, signals the parasite to start sexual development. However, enzymes related to lipid metabolism and the metabolic pathways involved have not been studied in detail yet. Here we show that a newly identified lipoxygenase in *T. gondii* (TgLOX1), has a role that goes beyond initiating sexual reproduction, negatively affecting parasite fitness in vivo when it is knocked out. Initial characterization of parental, KO, and complement ME49 strains on tissue culture did not reveal any phenotypical differences. When mice were infected to obtain brain cysts, animals injected with the KO strain were able to completely clear the infection after 3 days, making it impossible to obtain cysts. Further analysis of the localization of TgLOX1 revealed that, in tissue culture, the enzyme was found in the cytoplasm. However, when parasites were injected into mice and collected after 3 days, TgLOX1 was found in the parasite membrane of extracellular parasites or in the cytoplasm of host immune cells of intracellular parasites. These results suggest that TgLOX1 alters the host immune response allowing the parasite to avoid detection and disseminate. We believe that our results will open the way to new studies into the parasite's lipidome. These results suggest that not only ROP, GRA, and micronemes are necessary to invade the host, but enzymes related to lipid metabolism are important as well.

P6. Modification of dsDNA break repair mechanisms in *Trypanosoma cruzi*

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The protozoan parasite *Trypanosoma cruzi* is the cause of Chagas disease, an underestimated and understudied Neglected Tropical Disease (NTD). Although, in the past decade, advances in the study of *T. cruzi* biology have been made with the advent of genetic engineering via CRISPR/Cas9, many limitations remain. In particular, the Microhomology-Mediated End Joining (MMEJ) pathway that *T. cruzi* uses to repair dsDNA breaks can result in deletions that extend into genes flanking CRISPR-targeted genes. *T. cruzi* appears to have lost the ability to repair double-strand breaks using the more common and more conservative Non-Homologous End Joining (NHEJ) pathway. However, related trypanosomatids such as *Angomonas deanei* retain the key proteins for the NHEJ pathway, suggesting this species as a possible source of genes for establishing NHEJ in *T. cruzi*. In order to confirm NHEJ in *A. deanei*, we first used Cas9-containing ribonucleoprotein complexes to endogenously tag the *A. deanei* GP72 surface glycoprotein with eGFP. We have previously shown that MMEJ in *T. cruzi* generates a highly specific 33bp deletion in eGFP. We will use this property to confirm the NHEJ repair mechanism in *A. deanei* and if confirmed, attempt to transfer critical missing components of this pathway (e.g. Ligase IV) from *A. deanei* to *T. cruzi*. Establishing NHEJ in *T. cruzi* may also require blocking the activity of genes required for MMEJ. This may be accomplished using recently discovered chemical inhibitors or by the knockout of genes such as polymerase-θ. Modifying the non-homologous dsDNA break repair in *T. cruzi* is a challenge that if overcome, would help in the development of an efficient CRISPR technique to study large gene families and a CRISPR screening library.

P7. Breaking out: Egress of malaria parasites requires a putative glycosyltransferase

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Malaria is a deadly disease caused by the apicomplexan parasite *Plasmodium*. *Plasmodium* asexual replication occurs in the red blood cell (RBC) and is what causes clinical symptoms of disease. In the RBC, *Plasmodium* moves through 3 developmental stages ending with schizogony. Life cycle completion and successful egress allows for exponential *Plasmodium* replication and RBC infection. We recently identified a glycosyltransferase (B3GLCT-like) that interacts with an essential *Plasmodium* ER chaperone (PfB3ER). In mammalian cells, B3GLCT-like proteins work in concert with protein O-fucosyltransferases (POFUT2) to modify thrombospondin-like repeats. Surprisingly, *P. falciparum* POFUT2 has been shown to be non-essential while PfB3ER is predicted to be essential in the asexual stages. To investigate PfB3ER function, we employed CRISPR/Cas9 gene editing to create conditional mutants utilizing the TetR-DOZI aptamer system. We show that PfB3ER localizes to the ER, contrary to literature suggesting PfB3ER is exported to the host RBC, and that PfB3ER is primarily expressed during schizogony. Further, we show that PfB3ER is essential for the asexual replication of *P. falciparum*. Our data suggest that knockdown of PfB3ER leads to a prolonged asexual life cycle that takes about 64 hours instead of 48 hours. We do not observe any morphological defects upon knockdown and the extended asexual life cycle results in the formation of morphologically normal schizonts that fail to egress. Transcriptomic data corroborate the observed prolonged asexual life cycle. With ongoing studies, we are testing PfB3ER function during egress, and studying PfB3ER glycosyltransferase activity.

P8. The role of Phe-337 in *Trypanosoma cruzi* glucokinase: Thermodynamic evaluation on the binding interaction of glucosamine-based inhibitors

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Throughout the world there are 6 – 7 million people affected by Chagas' disease, a neglected tropical disease caused by the parasite *Trypanosoma cruzi*. This protozoan microorganism has a glucokinase (TcGlcK) that is considered to be a potential drug-target based on its role of producing G6P, a key metabolic intermediate. An urgent need exists in identifying new therapeutics as the clinically available options such as benznidazole and nifurtimox give rise to undesirable side effects. In the past decade, multiple studies have demonstrated that various TcGlcK inhibitors also act as anti-*T. cruzi* compounds with a tight correlation. In an ongoing effort to identify stronger TcGlcK competitive inhibitors, we structurally examined the active site of the enzyme in complex with carboxybenzyl glucosamine (CBZ-GlcN) due to its enhanced inhibition profile. Through enzyme – inhibitor biochemical assays, CBZ-GlcN was reported to have a Ki of 0.71 μM, which appears to be rooted in an important pi-pi stacking interaction with the side chain of Phe-337 and the benzyl group of CBZ-GlcN. To test this hypothesis, a series of mutations were made to TcGlcK at position Phe-337, including F337L, F337V, and F337A, in order to progressively diminish any substantial intermolecular interactions. Biochemical studies used in this project were X-ray crystallography, isothermal titration calorimetry, and enzyme – inhibitor kinetics followed by a thermodynamic assessment in binding between wild-type and the mutant forms. Understanding the role of Phe-337 provides insight into the strategic design of glucokinase competitive inhibitors and can be extended to other glucokinases from other pathogenic microorganisms.

P9. ClinEpiDB.org: An open access platform for sharing and exploring global health datasets

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Data sharing from epidemiology studies, clinical trials, and implementation research promotes transparency and accelerates discovery while providing opportunities for innovation and collaboration among global health researchers. VEuPathDB's clinical epidemiological database ClinEpiDB.org is an open-access, online resource that allows global health researchers to not only meet, but surpass, the requirements of journals and funders for data sharing by integrating study data with standardized ontologies to make data more easily reusable. Study teams have full ownership of data and control the level of data access. ClinEpiDB's newly rebuilt exploratory data analysis platform facilitates powerful data explorations right in the browser. An interactive codebook helps to browse variables, subset data, and examine frequency distributions and variable associations. New innovations include "Featured variables" and the ability to star favorite variables, which make it easy to identify variables that are key to understanding the study data and keep track of variables of interest. Data visualization tools offer additional ways to explore study data online by allowing users to stratify by other variables, zoom within the plots, add best fit lines or calculate basic statistics, and more. Users can choose whether to download a subset or all of the data, or request access directly within the browser if required by the original study team. Over 87% of data access requests are approved. In four years of operation, ClinEpiDB has integrated data on >1 million participants from >30 studies, and includes datasets from the MAL-ED, GEMS, and WASH Benefits enteric disease projects, SCORE schistosomiasis project, and ICEMR malaria studies, among others. Additional studies and new tools are released every two months. The ClinEpiDB resource provides an intuitive interface to access epidemiological data and will continue to grow with integration of new datasets, tool development, and user outreach and education.

P10. Elucidating the mechanism of resistance of β-carboline derivatives

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Malaria is a deadly disease that affects nearly half the world's population. While we currently have preventative and curative measures to reduce parasite burden, drug resistance has been identified to all clinically approved antimalarials. Because of increasing antimalarial resistance, there is an urgent need to discover new targets and chemotherapies potent against malaria, in addition to understanding mechanisms of resistance to antimalarials. The multidrug resistance protein (PfMDR1) is a common mechanism of resistance to chemotherapies against malaria. Despite the prevalence of PfMDR1 mutations, we do not fully understand how PfMDR1 modulates resistance in the malaria parasite. In our lab, we have discovered a novel enantiopure benzofuran-2-carboxamide of 1-aryltetrahydro-β (beta)-carboline compound, PRC1590, that is potent against multiple strains of *Plasmodium falciparum*. Through in vitro evolution of resistance to PRC1590 paired with whole genome sequencing and cross resistance screening, we have identified that PRC1590 resistance is mediated by a single nucleotide polymorphism (SNP) located on pfmdr1. Moreover, other strains of *P. falciparum* carrying SNPs on the pfmdr1 gene also maintain resistance to PRC1590. We are continuing to characterize resistance to PRC1590 to aid in rational drug design, in addition to using PRC1590 as a probe to better elucidate PfMDR1 as a resistance mechanism.

P11. Identification of beta-carboline derivatives active against quiescent artemisinin-resistant *Plasmodium falciparum* parasites

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Malaria is a devastating disease that caused approximately 600,000 deaths in 2020 worldwide. Cases of malaria have increased from previous years in part by the quick development of resistance to current antimalarials. Due to the rising resistance to current antimalarial drugs such as chloroquine and artemisinin, there is an urgent need to discover and develop new chemotherapeutic agents that engage new targets in the malaria parasite. This research focuses on a novel antimalarial with a unique beta-carboline scaffold known as PRC1584. It is known that exposure to dihydroartemisinin (DHA) induces a quiescent state in *Plasmodium falciparum* ring stage. Quiescence is a mechanism of *Plasmodium* survival especially from drug treatment. This phenomenon increases the risk of clinical failures following artemisinin-based combination therapies by slowing parasite clearance and allowing the selection of parasites resistant to partner drugs. We investigated if short exposure of PRC1584 also induces quiescent and/or kills the proliferating ring stage and if PRC1584 has activity against DHA-induced quiescent ring stage in the presence or absence of DHA-resistance. We used the ring survival assay (RSA) and the quiescent-stage survival assay (QSA) to assess the antiplasmodial activity of PRC1584 and its analogs in the presence and absence of DHA resistance. Our studies revealed that only 8 hours of exposure to PRC1584 kills both the proliferating ring stage and the DHA-induced quiescent rings of *P. falciparum* independently of the presence of DHA-resistance. In addition, we are using these assays to guide optimization of this series as preclinical leads. Altogether, these results revealed that PRC1584 displays a fast-killing profile and that it may act through a novel mechanism of action. Identifying if new antimalarials may also facilitate the development of quiescent-ring stage is extremely important, as this could result in recrudescence and treatment failure.

P12. The regulation of proteolytic cascade in egress of *Plasmodium falciparum*

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A severe worldwide public health concern, malaria is caused by parasites in the genus *Plasmodium*. *P. falciparum* is responsible for almost all the deaths. At the end of proliferation, mature merozoite parasites must be released first from a parasitophorous vacuole membrane (PVM) and then from the red blood cell membrane (RBCM). The *P. falciparum* endoplasmic reticulum resident calcium-binding protein or PfERC is required for parasite egress by regulating the aspartic protease's maturation Plasmepsin X (PMX) in the secretory compartment called exonemes. While In vitro studies indicate that PMX matures via autoproteolysis and its activity requires acidic pH, no published data suggests that exonemes are acidic in nature. In addition, relatively little is known about PMX maturation. Using a pH-sensitive green fluorescent protein (GFP) known as super-ecliptic pHlourin (SEP) to the endogenous locus of PMX (PMXSEP) in PfERC mutants, we showed PMXSEP localizes to exonemes and partially co-localizes with PfERC. In immature schizonts, PMXSEP has been observed. Loss of fluorescence over time indicates that the exonemes become more acidic. In mature schizonts, the PMXSEP are fluorescence which suggests they are released from the exonemes. Egress inhibitor inhibits exocytosis resulting in no fluorescence events observed. Our study showed the first observation of organelle exocytosis minutes before egress.

P13. Comparative analyses of a newly sequenced and annotated *C. meleagridis* genome

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Cryptosporidium spp. are medically and scientifically relevant protozoan parasites that cause severe diarrheal illness in infants and immunosuppressed populations as well as other animals. It is the most common cause of recreational waterborne disease in the United States. Although most human *Cryptosporidium* infections are caused by *C .parvum* and/or *C. hominis*, there are several other human-infecting species including *C. meleagridis*, with a higher prevalence in developing countries. *C. baileyi* is uniquely avian infecting species. Recently, a new *C. parvum* IOWA-ATCC genome assembly generated from Pacific Biosciences (PacBio) long-reads and new annotation were published. Here, we sequenced and assembled a new long-read genome sequence for *C. meleagridis*, which infects both avians and humans, using a unique Whole Genome Amplification (WGA) and long-read Nanopore approach. We generated an annotation and performed a comparative analysis with the most recent *C. parvum*, *C. hominis* and *C. baileyi* genome sequences and annotation. The new *C. meleagridis* and *C. parvum* genome assemblies are gap free. Comparative analyses of the genome sequences of *C. meleagridis* with *C. parvum* showed that the genomic sequences are highly syntenic. A comparison of annotated proteins in *C. meleagridis* and *C. parvum* surprisingly revealed near perfect orthology. Our study highlights the similarities and differences between *C. parvum* and *C. meleagridis* and adds *C. hominis* and *C. baileyi* as comparators for human- and avian-infecting species. This comparative study reveals few differences between *C. parvum*, *C. hominis*, and *C. meleagridis* but shows significant differences between *C. meleagridis* and *C. baileyi*.

P14. VEuPathDB: Omics support for the global parasite, vector and fungal research communities

Susanne Warrenfeltz[1] … on behalf of the entire VEuPathDB Project

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VEuPathDB (https://veupathdb.org), is a family of free, online bioinformatics resources supporting >500 species including protozoan parasites, fungi and oomycetes, arthropod vectors and selected host species. VEuPathDB resources facilitate the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. Specialized analytical or computational skills are not required since tools are offered in a friendly web-interface and supported by an email help desk, video tutorials, webinars, and social media. Available data types include genome sequence and population-level variation data; manually-curated and automatically generated annotation; epigenetic, transcriptomic and proteomic data; and pathway information. In addition, geospatially resolved vector surveillance data is available in the VectorBase tool called MapVEu. To take advantage of data from other species, a phylogenetic framework facilitates cross-species functional inference via orthology. In addition, a Galaxy interface provides a bioinformatics platform for privately analyzing your own large scale data and porting your results to VEuPathDB for comparisons with public data. VEuPathDB's comprehensive data mining resources offer valuable in silico hypothesis development and testing, so that end users can answer questions concerning expression levels and timing, domain presence, gene model integrity and genetic variation before venturing into the laboratory. VEuPathDB is one of two NIAID-supported Bioinformatics Resource Centers, and receives additional support from the Wellcome Trust, the Bill & Melinda Gates Foundation. Please email help@veupathdb.org with questions or suggestions.

P15. Characterizing the *T. gondii* homolog of ATPase Inhibitory Factor 1 (IF1)

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Apicomplexan parasites cause several deadly and debilitating diseases around the world including malaria, cryptosporidiosis, and toxoplasmosis. Recent studies of apicomplexan biology have revealed that several features of their mitochondria diverge significantly from their human host. An example of this is the ATP synthase: over half of the subunits in the apicomplexan ATP synthase have no known homologs outside the phylum. Despite the divergence in subunit composition, it was recently determined that a structural homolog of a conserved eukaryotic ATP synthase inhibitor, ATPase Inhibitory Factor 1 (IF1), binds the *T. gondii* enzyme. In other eukaryotes, IF1 inhibits the ATP synthase to reprogram metabolism in favor of glycolysis and can facilitate ATP synthase dimerization. Additionally, IF1-mediated inhibition of the ATP synthase can activate cytoprotective pathways that reduce mitochondrial stress: a process termed mitohormesis. Nonetheless, it is unknown whether the structural homolog of IF1 identified in *T. gondii* (TgIF1) functions similarly. To investigate the role of TgIF1 we have created parasite strains in which the gene is endogenously tagged, knocked out, or exogenously overexpressed. Interestingly, TgIF1 overexpression results in the stable formation of a high molecular weight oligomer. Further, plaque assays have illustrated that IF1 knockout or overexpression does not significantly impact the intracellular replication of *T. gondii* tachyzoites under normal growth conditions. While IF1 overexpression has been shown to increase ATP synthase dimerization in other organisms, our native gel electrophoresis studies illustrate that neither TgIF1 knockout nor overexpression impact ATP synthase dimerization. We are currently working to determine how TgIF1 may regulate parasite metabolism and whether it can initiate mitohormesis in response to stress. These investigations will provide novel insight into the ways that the apicomplexan ATP synthase is regulated and how these parasites adapt to the varying environments faced throughout their complex life cycles.

P16. Function of conserved transmembrane proteins in *Plasmodium* egress

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Malaria is a serious public health issue that affects millions around the world and is especially detrimental to children under the age of 5. During infection, *Plasmodium* parasites invade red blood cells (RBCs), multiplying and expanding within the bloodstream and causing serious clinical disease. After replicating within RBCs, *Plasmodium falciparum* will egress from the host cell in the form of 16-32 infectious daughter merozoites which reinvade surrounding cells. *Plasmodium* egress is regulated by a poorly understood signal-dependent pathway that commits the parasite to host cell rupture during schizogony. Recent work has identified that an ER-resident calcium binding protein (PfERC) is an essential regulator of *Plasmodium* egress. Using quantitative proteomics, we identified proteins interacting with PfERC (PIEs) as candidates for further study based on essentiality and peak expression during schizogony. Our work is focused on discovering the function of two conserved membrane proteins, Pf3D7_1117400 (PIE1) and Pf3D7_0522600 (PIE2). PIE1 has domain architecture similar to soluble NSF attachment protein receptor (SNARE) proteins, which mediate intracellular membrane fusion in many eukaryotes. This, along with peak expression during schizogony and interaction with PfERC leads us to believe PIE1 has a role in intracellular membrane fusion of secretory organelles during egress. PIE2 has sequence homology to cation transporters which suggests they could function in proton transport and pH regulation during schizogony. Using CRISPR/Cas9 genetic engineering to incorporate the PfDOZI-TetR-based knockdown system, we have manipulated parasites to generate conditional mutants to study how these two proteins function. Using these knockdown mutants, we aim to use established assays to determine essentiality, localization, and role in the signal-dependent egress pathway. Because proliferation of the parasites relies on egress of daughter merozoites, recent research has focused on unveiling the mechanisms behind this process in order to find novel antimalarial drug targets.

P17. Malaria@TheSporoCore, where mosquitoes are more than just a flying syringe

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Mosquitoes vector some of the most devastating diseases of humans and wildlife. Amongst vector-borne diseases of humans, malaria is probably the most virulent, and is caused by several species of unicellular, parasitic eukaryotes in the genus *Plasmodium*. Managing the transmission of this parasite by their *Anopheles* vectors reduced the global incidence of malaria to a historic low; indeed, this reduction changed the narrative from control to elimination, and even eradication. To reach these goals, we need to refine, as well as develop new strategies to control transmission of *Plasmodium* parasites by mosquitoes. However, propagating *Plasmodium*-infected mosquitoes in the laboratory is challenging due to the complex life history of the parasite, and often require personnel dedicated to the task. At the SporoCore, we meet this need by generating *Plasmodium*-infected mosquitoes for distribution to research groups here at the UGA and at other non-profit institutions across the USA. Since officially opening its doors in August 2020, we have distributed ~150,000 An. stephensi mosquitoes, primarily infected with rodent strains of *Plasmodium*. In addition to describing the biological framework underpinning the facility, our poster provides an overview of the various applications currently enabled by these mosquitoes. At the same time, we showcase research conducted by graduate and undergraduate students at the SporoCore, where we describe how parasite life-history is shaped by its interactions with the vector and vertebrate hosts, with applications ranging from its consequences for current and novel anti-parasite interventions, to the effect of climate change and urbanization. As such, our hope is to initiate novel collaborations, and solicit input from the research community at the UGA regarding our potential to expand and/or integrate into other avenues of basic and translation research into parasite biology.

P18. Identification of new genes and pathways contributing to ivermectin hypersensitivity and resistance in *C. elegans* based on transcriptomics data from *B. malayi*

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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 *Brugia malayi* adults and microfilariae after treatment of infected gerbils. Forty-four of these DEG had *C. elegans* orthologs available as mutant strains through the *C. elegans* Genetics Center. 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. Overall, we identified eleven strong candidate genes as altering ivermectin sensitivity in at least one assay. These include genes related to previously implicated mechanisms of resistance such as: che-12 (e1812), a gene required for the normal structure and function of distal ciliary structures on amphid neurons and a known dye filling defective mutant (a phenotype associated with ivermectin resistance); and wht-4 (ok1007), a gene encoding an ABC transporter, a family of genes in which mutations have been associated with ivermectin toxicity in animals and overexpression with resistance in helminths. We also identified genes involved in pathways not previously associated with ivermectin's mechanism of action or resistance such as: tyr-2 (ok1363), a dopachrome isomerase involved in eumelanin synthesis; and lips-7 (ok3110), a lipase involved in lipid metabolism and longevity.

P19. Roles of the microbiome in immune system function in kissing bugs

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Kissing bugs, the main vector of Chagas Disease, are obligately hematophagous and must consume a blood meal before each nymphal molt. Kissing bugs house a limited diversity of microorganisms in their gut. These bacteria were deemed to be beneficial to the insect as the removal of them via surface sterilization of eggs results in increased developmental times, increased mortality, and failure to reach the reproductive adulthood stage. It is hypothesized that these bacteria provide essential B vitamins that are not abundant in vertebrate blood, but little is known about other key physiological process that the microbiome influences in kissing bugs. To investigate how the microbiome impacts kissing bug immunity, we analyzed host immune response in bacteria-free insects (axenic) and insects with defined microbiomes (gnotobiotic). Axenic insects demonstrated decreased immune gene expression after being immune challenged compared to insects with a microbiome. Axenic bugs were also more likely to die after immune challenge compared to their gnotobiotic counterparts. Microbiome composition also influenced immune system function as the supplementation of a non-symbiotic bacteria partially rescued immune activity compared to axenic nymphs.

P20. Single-molecule full-length Iso-seq data reveal and help explain *Cryptosporidium parvum*'s transcriptional landscape

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Cryptosporidium parvum is an apicomplexan protist parasite. Cryptosporidiosis, which is caused by this parasite is one of the leading causes of diarrhea in infants globally. *C. parvum* has a compact 9.1 Mb genome sequence of which 80% is coding sequence. *C. parvum* has lost numerous pathways including de novo DNA synthesis for all nucleotides and certain eukaryotic gene regulatory systems such as the RNA interference pathways (Dicer and Argonaute). Previous work from our lab identified long noncoding RNA (lncRNA) in *C. parvum*. We hypothesize that lncRNA may play important roles in regulating gene expression during the parasite lifecycle. Here we utilized single-molecule full-length RNA sequencing, known as PacBio Iso-seq, to characterize the RNA landscape. Iso-seq is a 3rd generation long read RNA sequencing adaptation of PacBio's Single Molecule Real-Time (SMRT) II technology. Iso-seq generates poly-A enriched full-length highly accurate transcripts, and it is inherently strand specific. Our Iso-seq results allowed us to annotate 5-prime and 3-prime Untranslated regions (UTRs) of protein coding genes in *C. parvum*. Analysis of, and manual annotation with Iso-seq have also increased the number of identified lncRNA genes by 50%. Analysis shows the majority of *C. parvum*'s lncRNA are anti-sense. Additionally, these antisense lncRNA's predominantly occupy the 3-prime ends of protein coding genes. The Iso-seq based gene expression profiles also revealed differential RNA turn over patterns due to accurate capture of transcription start sites (TSSs) and transcription end sites (TES) for each gene. Even though a relatively small portion of *C. parvum*'s protein coding genes contain introns, Iso-seq revealed alternative splicing patterns.

P21. Analysis of the PJW/PP1 complex involved in RNA Pol II transcription termination in trypanosomes

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Pol II termination of protein-encoding genes in eukaryotes is directly linked to 3'-end formation where cleavage of the nascent transcript at the poly(A) site provides access for the 5'-3' RNA exonuclease that eventually leads to dissociation of the polymerase from the DNA template. The genomes of kinetoplastids are organized into polycistronic transcription units and therefore, somehow decouple termination from 3'-end formation of individual genes and allow termination at the end of the array. We have previously shown that termination sites in kinetoplastids contain a novel modified DNA base (base J, β-D-glucosyl-hydroxymethyluracil) where the loss of J leads to termination defects and expression of downstream genes. We recently identified a PJW/PP1 complex in *Leishmania* containing a J-binding protein (JBP3), PP1 phosphatase 1, and the PP1 interactive-regulatory protein (PNUTS). Our analysis of the complex in *T. brucei* and *L. major* suggests it regulates transcription termination by recruitment to termination sites via JBP3-base J interactions and dephosphorylation of specific proteins by PP1. PNUTS presumably provides a scaffolding function for the entire complex and regulates PP1 substrate specificity. To address this model, we have devised a chromatin binding assay to examine the role of base J and JBP3 in PJW/PP1 complex association at Pol II termination sites in vivo. Inhibition of base J synthesis using DMOG leads to only ~20% dissociation of JBP3 and PNUTS at 100 mM NaCl. Suggesting base J is not the only component of PJW/PP1 complex: chromatin associations in *T. brucei.* One possibility we will explore is H3v that co-localizes with J at termination sites. Chromatin binding analyses combined with RNAi are also addressing the function of PNUTS as a scaffold factor and the functions of two Tb PP1 isoforms in the termination mechanism. Results from these experiments will be discussed.

P22. Understanding the role of bacterial symbiont *R. rhodnii* in kissing bug lipid metabolic physiology

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Bacterial symbionts are known to play an essential role in development and reproduction of many insect hosts, especially through promotion of host metabolism and nutrient provisioning. Axenic, or germ-free, insects exhibit nutrient deficiencies and inability to metabolize lipids and carbohydrates, which further impacts their ability to undergo normal development. Thus, axenic insects experience significantly longer developmental times or complete developmental arrest. In blood-feeding or hematophagous insects, symbionts are important for provisioning B vitamins, which are not believed to be highly abundant in vertebrate blood. These B vitamins are important cofactors for many metabolic pathways, and are unable to be synthesized by animals. B vitamin provisioning was also thought to be the primary role of gut-symbiont *Rhodococcus rhodnii* in its kissing bug host, *Rhodnius prolixus*. To expand our knowledge of this key host-symbiont relationship, we sought to investigate the role of *R. rhodnii* in kissing bug lipid metabolism. We conducted a 1st instar gut transcriptome comparing axenic nymphs and nymphs singly inoculated with *R. rhodnii* (gnotobiotic) and identified 11 highly differentially expressed lipid metabolic genes, most of which were upregulated in gnotobiotic nymphs. Furthermore, we found that gnotobiotic 4th instar nymphs have significantly higher lipid stores than axenic bugs. Our findings demonstrate that *R. rhodnii* likely has additional roles in bugs outside of B vitamin provisioning.

P23. Phosphoinositide phospholipase C is essential for the infective stages of *Trypanosoma cruzi* but is not involved in the synthesis of inositol pyrophosphates

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Phosphoinositide phospholipase C (PI-PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to generate the second messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). In mammalian cells, IP3 gates the IP3 receptor, and stimulates Ca^{2+} release, while DAG remains in the membrane and stimulates protein kinase C. PI-PLC plays an essential role in cell signaling and it regulates many processes, such as cell division, secretion, and differentiation in these cells. In yeasts, IP3 can be further phosphorylated at different hydroxyl positions producing IP4 and IP5 by inositol phosphate multikinase (Arg82), and IP6 by inositolpentakisphosphate kinase (IPK1), producing the fully phosphorylated form known as inositol hexakisphosphate (IP6) or phytic acid, which can be a precursor of the inositol pyrophosphates. Inositol pyrophosphates are characterized by the presence of single (PP-IP4 and PP-IP5) or double (PP2-IP3 and PP2-IP4) pyrophosphate moieties linked at different positions of the myo-inositol backbone. Here, we report the use of a CRISPR/Cas9-based strategy to generate TcPI-PLC knockout and complemented epimastigotes, as confirmed by PCR, Southern blot, and RT-PCR analyses. TcPI-PLC-KO epimastigotes were viable and able to undergo metacyclogenesis. However, TcPI-PLC-KO trypomastigotes have a reduced capacity to invade host cells and to replicate within them as amastigotes. Capillary electrophoresis-electrospray ionization-mass spectrometry analyses of inositol pyrophosphates revealed that their synthesis was not affected in TcPI-PLC-KO epimastigotes, indicating that inositol pyrophosphate synthesis does not depend on the formation of IP3 by TcPI-PLC and suggesting the presence of a lipid-independent pathway involved in their synthesis. In conclusion, our data shows that TcPI-PLC is dispensable in epimastigotes, but it is critical for the virulence of infective stages. In addition, deletion of TcPI-PLC does not affect the inositol pyrophosphate synthesis pathway.

P24. Protein disulfide isomerase of *Toxoplasma gondii*

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Protein disulfide isomerases (PDI) are endoplasmic reticulum (ER) enzymes that catalyze the breakage and formation of disulfide bonds between cysteine residues in proteins in order to chaperone protein folding and regulate ER Ca^{2+} homeostasis. In mammals the PDI family, which is a subgroup of the thioredoxin superfamily of proteins, is composed of 21 different proteins. Members of the PDI family are functionally diverse but all contain at least 1 thioredoxin-like domain. Those proteins, which carry out disulfide bond breakage and/or formation, have canonical CXXC motifs that function as the active site for oxidative protein folding. *Toxoplasma gondii* is an obligate intracellular parasite that belongs to the phylum Apicomplexa. The role of PDI proteins in the *Toxoplasma gondii* ER is poorly understood. In this work we generated a conditional mutant with an N-terminal tag for one of the annotated *T. gondii* PDI (TgPDI) (TGGT1_211680) using the CRISPR/Cas9 system. Analysis of the mutant showed that TgPDI is essential for the *T. gondii* lytic cycle. We also demonstrated the redox ability of the protein using the redox-active crosslinker divinyl sulfone (DVSF). Mass spectrometry data from TgPDI co-Immunoprecipitations utilizing DVSF revealed enrichment of other redox active proteins, proteins involved in GPI anchor biosynthesis, and microneme and rhoptry proteins. This work highlights important functions of the ER in protein secretion and maturation.

P25. Characterization of two methyltransferases in *Toxoplasma gondii* ubiquinone biosynthesis pathway

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Toxoplasma gondii is a protozoan parasite that causes one of five neglected parasitic infections in the United States, and toxoplasmosis is fatal in immunocompromised patients. However, current medicines used to treat toxoplasmosis have toxic side effects and require long term treatment for immunocompromised patients. Therefore, medicines with enhanced efficacy that target unique metabolic steps of *T. gondii* is of great interest to improve toxoplasmosis therapy. The ubiquinone biosynthesis pathway produces Coenzyme Q (UQ), which functions in the mitochondrial electron transport chain shuttling electrons from complex I or II to complex III. The UQ synthesis in *T. gondii* appears to diverge from human, and several enzymatic steps could represent novel therapeutic targets. In *Saccharomyces cerevisiae*, several Coq enzymes form a complex called ubiquinone synthome, which has not been characterized in *T. gondii*. Moreover, very little is known about this pathway in *T. gondii* and other apicomplexan parasites. We used conditional gene disruption along with various functional analysis assays to investigate the role of two methylation activities, TgCoq3 and TgCoq5, in mitochondrial membrane potential and mitochondrial oxygen consumption. We successfully complemented the yeast Coq3 knockout strain with TgCoq3 and found that TgCoq3 can be utilized in yeast to partially rescue the growth defect. We created the C-terminal tagged cell lines with the TurboID biotin ligase and demonstrated that TurboID was able to enrich mitochondrial proteins. The future goal of this project is to identify specific interactors of these two proteins using Co-IPs and specific inhibitors that could also impact parasite growth.

P26. Induced In vitro sexual commitment of *Plasmodium cynomolgi*

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Plasmodium vivax is the most geographically widespread malaria species to infect man, yet our understanding of its unique biology has been hindered by a lack of in-vitro culture systems and access to infected human samples. To circumvent this issue, phylogenetically related species such as *Plasmodium cynomolgi* have been historically utilized in non-human primate models to further our understanding of *P. vivax* infections. Recently, *P. cynomolgi* was successfully adapted to long-term in-vitro culture, expanding the utility of this model species outside of the non-human primate model. Gametocytes, sexually committed parasites capable of mosquito infection, are relatively uncharacterized and essential for mosquito infection, making them an ideal target for in- vitro study. Previous reports indicate *Plasmodium falciparum* and *Plasmodium berghei* parasites that overexpress gametocyte associated genes Api-AP2G (AP2-G) and Gametocyte Development protein 1 (GDV1) are capable of in-vitro sexual commitment, yet this has not been accomplished in *P. cynomolgi*. However, transcriptional and genomic studies indicate the process of sexual commitment is likely conserved within the *Plasmodium* genus. To gain greater insight into the mechanism of sexual commitment in *P. vivax*-like parasites, we are currently generating molecular tools to overexpress *P. cynomolgi*-specific homologues to AP2-G and GDV1 within in-vitro cultured parasites. We aim to utilize both the centromere-containing overexpression plasmid, pCyCEN, as well as CRISPR/Cas9 to achieve our goals. In-vitro cultured *P. cynomolgi* will be transformed utilizing these tools to generate mutant parasites capable of sexual commitment. Isolated mutants will then be tested for mosquito infectivity via standard membrane feeding assay. This work will increase our understanding of sexual commitment in *P. vivax*-like parasites while simultaneously generating a valuable tool for future studies.

P27. Sweet danger: Protein glycosylation in human malaria parasites

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Plasmodium parasites are the causative agent of malaria, and infection by one species, *P. falciparum*, caused more than 400,000 deaths in 2019. All clinical malaria symptoms, up to and including death, are a direct result of the parasite's asexual replication within red blood cells (RBCs) in a 48-hour cycle. To build a suitable habitat for growth, the malaria parasite completely transforms the host cell, making changes that enable immune evasion and the favorable movement of nutrients. This essential remodeling process requires the export of hundreds of parasite proteins to the host cell, each of which must be modified in the endoplasmic reticulum to be fully functional. One such modification is the addition of complex sugars to the side chains of asparagine residues on target proteins, a process called asparagine linked glycosylation (N-glycosylation). While this ubiquitous protein modification has been demonstrated to be essential for protein function and trafficking in model organisms, its importance in divergent eukaryotes such as *Plasmodium* spp. is both unclear and understudied. The highly conserved oligosaccharyltransferase (OST) complex is the enzyme that transfers a glycans to the consensus acceptor site N-X-S/T (X≠P) on nascent proteins, and there is a clear ortholog of the OST catalytic subunit STT3 in the *P. falciparum* genome (PF3D7_1116600, PfSTT3). Based the essential function of N-glycosylation in other eukaryotes, we hypothesize that the STT3-catalysed N-glycosylation of exported proteins is required for the asexual stage of *P. falciparum*. We will test this hypothesis by generating parasites in which the expression of PfSTT3 can be turned off in a controlled manner (termed PfSTT3apt). We are using these conditional mutants to determine if PfSTT3 is required for parasite survival, and if its enzymatic activity is required for protein trafficking. These mutant parasites will enable us to elucidate the localization, essentiality, and function of PfSTT3, and thus illuminate its putative role in protein N-glycosylation in the parasite.

P28. Memory regulatory T cells offer protection from malaria reinfection

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Regulatory T cells (Tregs) are a subset of CD4 'helper' T cells that allow the host to maintain immune tolerance and homeostasis. Defined by the expression of transcription factor Foxp3, Tregs are known to expand and restrain pro-inflammatory immune responses in primary blood-stage malaria, while also impeding the effective control of the infection. Considering that people residing in malaria-endemic regions get repeated infections with *Plasmodium*, it is important to understand how memory Tregs influence immunity to such recurrent infections. To our surprise, individuals living in malaria-endemic regions with high pre-existing frequencies of memory Tregs exhibited lower parasitemia levels when naturally reinfected with *Plasmodium* in the subsequent malaria season. In agreement with this observation, in mice, selective ablation of memory Tregs resulted in loss of protection from reinfection and transfer of memory Tregs conferred better immunity to fresh *Plasmodium* infections in the recipients. These finding are in stark contrast to the expectations based on how Tregs function in primary malaria infection. We show that, upon *Plasmodium* re-infection, memory Tregs get transcriptionally reprogrammed into a new subsets of helper cells – T follicular helper (Tfh) cells – and localize to the germinal centers in the secondary lymphoid organs, potentially facilitating the generation of robust antibody responses. Our findings show that memory Tregs protect against recurrent blood-stage malaria by undergoing transcriptional and phenotypic reprogramming. These observations will have a significant impact on the design of anti-malaria strategies in the endemic areas, where reinfection make up the majority of malaria cases.

P29. Evaluation of carbazole derivatives as leads for human African trypanosomiasis drug development

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Human African trypanosomiasis (HAT) is caused by *Trypanosoma brucei* sp. Current drugs for HAT have some undesirable properties that accompanied with signs of emergence of drug resistance necessitates a search for new leads for treatment of HAT. Curaxin CBL0137 cured HAT in a mouse model of the disease, spurring a search for backup compounds while it is developed into a drug. Related carbazoles CBL0174 and CBL0187 possess anti-proliferative and delayed cytocidality (DCC) activity equivalent to CBL0137. Further they have desired intestinal permeability, solubility, and metabolic profile in vitro. From pharmacokinetic analysis, brain and plasma concentrations exceeded ten-times the 50% anti-proliferative concentration (EC50) against *T. brucei*. Surprisingly, in a mouse model of HAT CBL0174 and CBL0187 failed to control tissue load of *T. brucei*. Towards an understanding of reasons for failure of CBL0174 and CBL0187 in the mouse, we found that anti-proliferative activity of CBL0187 plummets four-fold when serum concentration in the culture medium is increased. Free plasma effective concentrations of both CBL0174 and CBL0187 were less than DCC99, (24-h), unlike that of CBL0137. These data are consistent with a principle that efficacy of these drugs in a mouse model of HAT requires effective plasma exposure that exceeds DCC99. These new concepts will be used to revise criteria for evaluation of leads in our anti-HAT drug development program.

P30. Oxygen-dependent regulation of F-box proteins in *Toxoplasma gondii*

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Parasites depend on a dynamic proteome to adapt to changing environments, and the Skp1/cullin-1/F-box protein/Rbx1 (SCF) family of E3 ubiquitin ligases contributes importantly to proteasome-mediated degradation. Here, we examine the role of the novel posttranslational glycosylation of Skp1 that depends on the generation of a hydroxyproline anchor by the oxygen-dependent prolyl hydroxylase PHYa, a homolog of the HIFα PHD2 oxygen-sensor of human host cells. Based on other work, PHYa is required for virulence by evading IFNγ -induced nutritional immunity. Strikingly, the representation of several putative F-box proteins (FBPs) is substantially reduced in the Skp1 interactome of PHYaΔ parasites. One of these, termed FBXO13, is a predicted lysyl hydroxylase related to the human JmjD6 oncogene except for the presence of an F-box domain. The abundance of FBXO13-HA tagged at its genetic locus was reduced in PHYaΔ parasites thus explaining reduced presence in the Skp1 interactome. A similar effect in glycosylation-mutant cells, and partial rescue by proteasomal inhibitors, supported the involvement of Skp1 and the SCF. The nucleocytoplasmic localization of neither Skp1 nor FBXO13 was affected by Skp1 modification. Similar effects were observed for FBXO14-HA, a cytoplasmic protein of unknown function that is found only in apicomplexans that possess PHYa suggesting a co-evolutionary relationship. In contrast, FBXO1 was not affected by PHYa. These findings are physiologically significant because similar effects on Skp1, FBXO13 and FBXO14 were observed in parasites reared on monolayers under 0.5% O_2. The dependence of FBP abundance on Skp1 modification likely contributes to the reduced virulence of PHYaΔ parasites, which in turn may result from impaired ability to use ambient O_2 levels as a locational signal.

P31. A *Toxoplasma gondii* calcium/proton exchanger and its role in regulating acidic Ca^{2+} stores and Ca^{2+} uptake by the endoplasmic reticulum

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Calcium (Ca^{2+}) signaling is a universally conserved process among eukaryotes. In *Toxoplasma gondii*, a rise in cytosolic Ca^{2+} impacts biological processes such as secretion of adhesive proteins, motility and invasion of and egress from host cells. These lytic cycle facets are essential for tissue disruption and spread of Toxoplasma, supporting a role for Ca^{2+} signaling in pathogenesis. We characterized a putative calcium proton exchanger, TgGT1_319550 (TgCAXL1), which belongs to a conserved family of transmembrane proteins predicted to be Ca^{2+}/proton exchangers that localize to the Golgi apparatus. We introduced a multiple HA tag at the C-terminus of TgCAXL1 and found that it localizes to the ER and Golgi apparatus. We used CRISPR-Cas9 to generate a clonal knockout mutant and found that TgCAXL1 is important for Ca^{2+} release from the ER and acidic stores, most likely the Golgi, causing for the mutant to be defective in invasion of host cells. We demonstrate that TgCAXL1 impacts sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity, suggesting a physiological interaction between both proteins. Yeast complementation validated the functional role of TgCAXL1 as a Ca^{2+}/proton exchanger. *T. gondii* intracellular pH measurements revealed that TgCAXL1 controls the cytosolic recovery from acidic stress. The function of SERCA is pH dependent, as alkaline pH levels drastically reduced SERCA activity and an acidic pH can stimulate it. We hypothesize that the misregulation of pH in the Golgi and ER that results from the absence of TgCAXL1, can affect Ca^{2+} homeostasis in these organelles and the function of Ca^{2+} signaling molecules, specifically the ER and SERCA . For the first time, we reveal the significance of the Golgi in modulating Ca^{2+} signaling in *T. gondii*.

P32. Single-cell RNA profiling of *Plasmodium vivax*-infected hepatocytes reveals parasite- and host- specific transcriptomic signatures and therapeutic targets

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The resilience of *P. vivax*—the most widely distributed malaria-causing agent in humans— is in part due to its ability to form dormant liver forms called hypnozoites. These forms are capable of activating weeks, months, or years after an initial mosquito bite and are responsible for relapsing episodes that maintain transmission. The molecular signatures of hypnozoites and the hepatocytes they infect remain poorly understood due to technical challenges associated with liver-stages infections. To overcome technical limitations, we perform single-cell RNA sequencing to characterize host- and parasite- transcriptomic signatures. Using an in vitro liver stage system, we profile >1,000 *P. vivax* liver forms and the hepatocytes they infect. On the parasite's side, we highlight distinct transcriptional signatures between schizonts and hypnozoites, and identify key differences in transcripts encoding for cellular fating RNA-binding proteins. On the host side, we uncover the transcriptional response of hepatocytes infected with either schizonts or hypnozoites. We show that while infection results in the enrichment of processes associated with energy metabolism and antioxidant stress response, the transcripts underlying these processes differ between schizont and hypnozoite infected cells. We also show that infection results in the down-regulation of pathways associated with host immune response, supporting the notion that *P. vivax* liver forms alter their host cell to remain undetected. Overall, our work offers insight into *P. vivax* biology—shedding light on transcriptome-wide signatures associated with the enigmatic hypnozoite—and reveals host- and parasite- markers that can serve as targets for new liver-stage therapeutics.

P33. The role of the phosphatidylinositol phospholipase C in the synthesis of inositol polyphosphates of *Toxoplasma gondi*i

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Inositol pyrophosphates (PP-IPs) are energy-rich signaling molecules involved in critical cellular functions such as vesicle trafficking, phosphate homeostasis and stress responses. PP-IPs are emerging as important regulators of cellular homeostasis and energy metabolism. The first enzyme involved in the synthesis of PP-IPs is the phosphatidylinositol phospholipase C (PI-PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG). IP3 is an important messenger and it is mainly known by its function in Ca^{2+} signaling. However, IP3 can be further metabolized to inositol octakisphosphate (IP8). The PP-IPs pathway has not been studied in *T. gondii*, however genes that encode three of the PP-IPs kinases are present in its genome. We analyzed the PP-IPs content of extracellular tachyzoites using perchloric acid extraction, titanium bead enrichment and capillary electrophoresis electrospray ionization mass spectrometry (CE-ESI). We detected all the products of the pathway from IP3 to IP8. We next investigated PP-IPs levels in the TgPI-PLC mutants and we found than downregulation of the TgPi-PLC impacts the PP-IPs pathway by significantly decreasing IP3, IP4, IP5 and IP6. Interestingly, PP-IPs depletion induced an increase in polyphosphate (poly-P) short chain levels. In addition, we characterized the inositol polyphosphate multikinase (TgIPMK) enzyme which catalyzes the conversion of IP3 to IP5. We determined the TgIMPK cytosolic localization in extracellular tachyzoites and demonstrated its activity in the inositol polyphosphate pathway, which is essential por parasites growth. Overall, we present a novel essential pathway in *T. gondii* with high potential as a drug target and the first evidence of the link between inositol pyrophosphates metabolism and poly-p synthesis.

P34. Epitopes in the GPI attachment signal peptide of *Trypanosoma cruzi* mucin proteins generate robust but delayed and nonprotective CD8+ T cell responses

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Infection with the protozoan parasite *Trypanosoma cruzi* elicits substantial CD8+ T cell responses that disproportionately target epitopes encoded in the large trans-sialidase (TS) gene family. Within the C57BL/6 infection model, a significant proportion (30-40%) of the *T. cruzi*-specific CD8+ T cell response targets two immunodominant TS epitopes, TSKb18 and TSKb20. However, both TS-specific CD8+ T cell responses are dispensable for immune control and TS-based vaccines have no demonstrable impact on parasite persistence, a determinant of disease. Besides TS, the specificity and protective capacity of CD8+ T cells that mediate immune control of *T. cruzi* infection is unknown. With the goal of identifying alternative CD8+ T cell targets, we designed and screened a representative set of genome-wide, in-silico predicted epitopes. Our screen identified a novel T cell epitope MUCKb25, found within mucin family proteins, the 3rd most expanded large gene family in *T. cruzi*. The MUCKb25 response was characterized by delayed kinetics, relative to TS-specific responses, and extensive cross-reactivity with a large number of endogenous epitope variants. Similar to TS-specific responses, the MUCKb25 response was dispensable for control of the infection and vaccination to generate MUCK-specific CD8+ T cells failed to confer protection. The lack of any apparent immune control mediated by MUCKb25-specific T cells was attributed to the fact that MUCKb25-specific T cells exhibit limited recognition of *T. cruzi*-infected host cells. Overall, these results indicate that a substantial proportion of the CD8+ T cell compartment in *T. cruzi*-infected mice is occupied by T cells with limited effector potential.