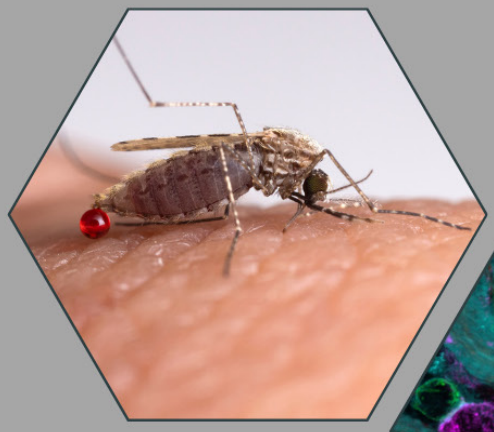
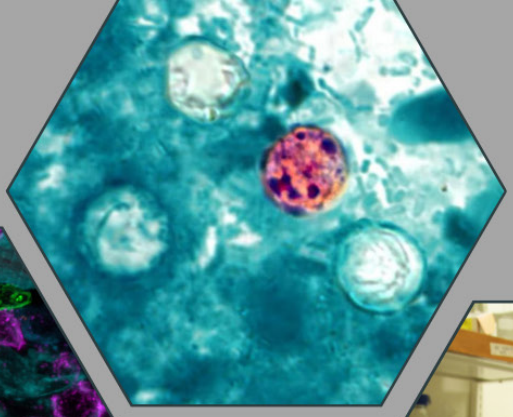
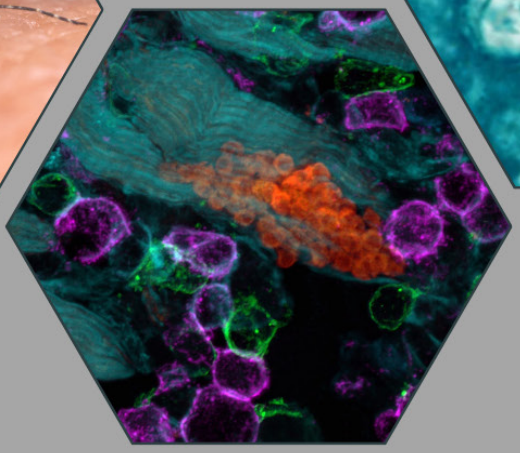
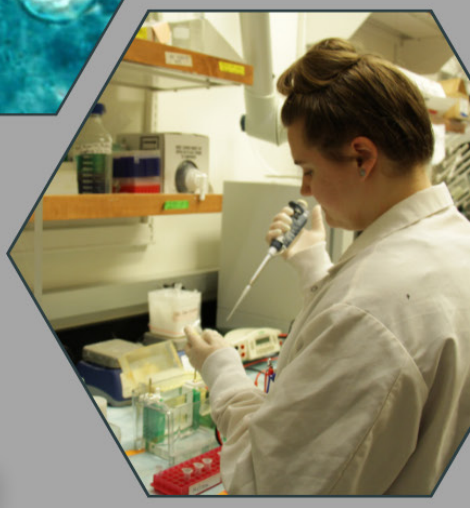
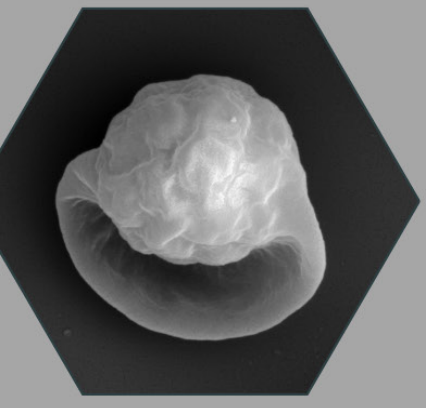
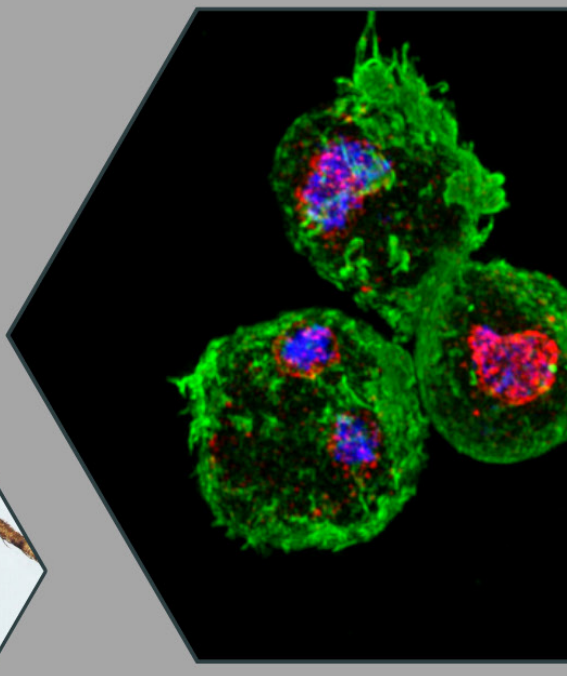
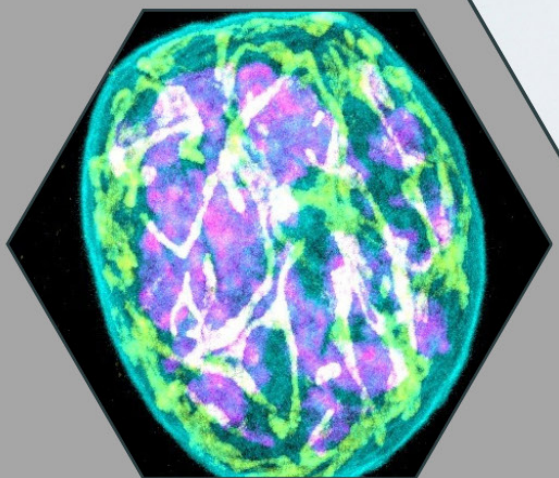





34th ANNUAL MOLECULAR PARASITOLOGY & VECTOR BIOLOGY SYMPOSIUM

May 12, 2025



Center for Tropical & Emerging Global Diseases
UNIVERSITY OF GEORGIA

Organizational support provided by:

Dennis Kyle, Director
David Peterson, Symposium Adviser
Annie Curry, Meeting Coordinator
Donna Huber, Publicity Manager
Angel Paradise, Travel Coordinator

http://ctegd.uga.edu
https://www. facebook.com/CTEGD
https://twitter.com/CTEGD
https://www.instagram.com/ctegd_uga/

To receive future announcements about the symposium, please sign up for our mailing list:
http://eepurl.com/81ZAX

Program

8:30 AM	Registration and Poster Set-up
9:00 AM	Opening Remarks: **Dennis Kyle**, Director of CTEGD
	SESSION 1 — Moderators: Fiifi Dadzie, Derek Huck, Kaelynn Parker
9:10 AM	**Justine Shiau**, Dept of Infectious Diseases, CTEGD, & Precision One Health, UGA *Plasmodium spp.* differential susceptibility to antimalarials targeting parasite mitochondria during the vector stages
9:30 AM	**Sabrina Pizarro**, EPIC & Department of Genetics and Biochemistry, Clemson University Exploration of putative sodium/proton exchangers in *Trypanosoma brucei*
9:50 AM	**Nana Charles-Chess**, CTEGD & Department of Cellular Biology, UGA Memort regulatory T cells protect against recurrent malaria
10:10 AM	**BREAK — POSTER VIEWING (even posters)**
	SESSION 2 — Moderators: Lena Argomaniz, Cierra Gladfelter, Fiifi Dadzie
10:50 AM	**Saniya Sabnis**, CTEGD, CVI, & Dept. of Infectious Diseases, UGA Humoral immunity leads to control of chronic *Plasmodium* infections
11:10 AM	**INTRODUCTION OF THE KEYNOTE SPEAKER**
11:15 AM	**Terrie Taylor**, Department of Osteopathic Medical Specialties, Michigan State University The pathogenesis of pediactric cerebral malaria: More pieces of the puzzle
12:15 PM	**LUNCH — POSTER VIEWING**
	SESSION 3 — Moderators: Victoria Mendiola, Corey Rennolds, Clyde Schmidt
1:15 PM	**Kaelynn Parker**, CTEGD & Dept. of Cellular Biology, UGA Same difference: An apicomplexan-specific extension in ATP synthase subunit f in *Toxoplasma gondii*
1:35 PM	**Joseph Dainis**, CTEGD & Department of Infectious Diseases, UGA Infection in the Collaborative Cross reveals differential susceptibility and resistance to *N. fowleri*
1:55 PM	**Derek Huck**, CTEGD & Department of Entomology, UGA Field-isolated bacteria support larval mosquito development under nutrient-limited conditions
2:15 PM	**Fiifi Agyabeng-Dadzie**, Department of Genetics, UGA Exploring the genome of a global pathogen: New insights into *Cryptosporidium parvum*
2:35 PM	**BREAK — POSTER VIEWING (odd posters)**
	SESSION 4 — Moderators: Cierra Gladfelter, Victoria Mendiola, Mac Sievert
3:15 PM	**Gonzalo Seminario-Mondejar**, Center for Tropical and Emerging Global Diseases, UGA Unraveling the enigmatic feeding apparatus of *Trypanosoma cruzi*
3:35 PM	**Alejandra Villegas Lopez**,University of Georgia A putative glycosyltransferase is required for *Plasmodium falciparu*m asexual development
3:55 PM	**INTRODUCTION OF THE EARLY CAREER SCHOLAR**
4:05 PM	**Fernanda Novais**, Dept. of Microbial Infection and Immunity, The Ohio State University, College of Medicine Hypoxia and CD8 T cells in cutaneous leishmaniasis
5:00 PM	Concluding Remarks: **Dennis Kyle**

Poster Presentations

P1 **Toya Tanner,** University of Georgia
Resistance unraveled: Dissecting drug sensitivity and resistance mechanisms in *Cryptococcus neoformans*

P2 **Surya Sekhar Pal**, Center for Inflammation, Immunity and Infection, Institute for Biomedical Science, Georgia State University
Enhanced neutralizing antibodies and protection against RSV by new pre-fusion mRNA and subunit protein combination vaccines in mice

P3 **Jillian McKeon**, EPIC & Department of Genetics and Biochemistry, Clemson University
Enolase inhibitors as therapeutic agents for *Naegleria fowleri* infection

P4 **Kenna Berg**, CTEGD & Department. of Infectious Diseases, CVM, UGA
Modulation of host cell apoptosis by secreted effectors during *Toxoplasma gondii* infection

P5 **Mayara Bertolini**, CTEGD & Department of Cellular Biology, UGA
Essential roles of vacuolar transporter chaperones 1 and 4 in polyphosphate metabolism and *T. cruzi* infectivity

P6 **Emily Bremers**, CTEGD & Department of Biochemistry and Molecular Biology, UGA
Stereospecific resistance to N2-acyl tetrahydro-β-carboline antimalarials is mediated by a PfMDR1 mutation that confers collateral drug sensitivity

P7 **Perla Vazquez**, Center for Tropical and Emerging Global Diseases, UGA
Conditioned media from differently virulent *Naegleria fowleri* differentially induces cytopathic effects over mammalian cell lines

P8 **Watcharatip Dedkhad**, University of Georgia
A *Plasmodium* transmembrane protein is essential for asexual segmentation of *Plasmodium falciparum*

P9 **Clyde Schmidt-Silva**, University of Georgia
Mechanism of antigen-presenting cell recruitment during liver-stage malaria

P10 **Hannah Teddleton**, Department of Animal Science, University of Tennessee, Knoxville
Parasite-resistant sheep exhibit metabolic efficiency after a *Haemonchus contortus* priming infection

P11 **Samantha Gunasekera**, Center for Tropical and Emerging Global Diseases, UGA
Uncovering the potential role of dsRNAs in *Cryptosporidium* gene regulation

P12 **Anissa Waller Del Valle**, CTEGD & Department of Cellular Biology, UGA
Development of a cell synchronization protocol for the brain-eating amoeba, *Naegleria fowleri*

P13 **Benjamin Hoffman**, Department of Cellular Biology, UGA
A nuclear protein with YqgF1 and SH2 domains regulates S-phase in *Trypanosoma bruce*i

P14 **Reagan Haney**, CTEGD & Department of Biochemistry and Molecular Biology, UGA
Identifying the mechanism of action of a novel antimalarial with collateral drug sensitivity associated with PfKelch13 C580Y mutation

P15 **Guozhong Huang**, CTEGD & Department of Cellular Biology, UGA
Chemical and genetic validation of an essential calcium entry channel of *Trypanosoma brucei* as a therapeutic target

P16 **Victoria Mendiola**, Center for Tropical and Emerging Global Diseases, UGA
Visualization and quantification of ART-induced dormant *P. falciparum* using cytoplasmic markers

P17 **Corey Rennolds**, CTEGD & Department of Genetics, UGA
Potency, plasticity, and diversity of stem cells in the rat tapeworm, *Hymenolepis diminuta*

P18 **Baihetiya Baierna**, CTEGD & Department of Cellular Biology UGA
Unique interactions between the succinate dehydrogenase and the ubiquinone biosynthesis complex in *Toxoplasma gondii*

P19 **Rafeed Turjya**, Institute of Bioinformatics UGA
The enigmatic mitochondrial genome of *Sarcocystis neurona*

P20 **Colm Roster**, EPIC & Department of Genetics and Biochemistry, Clemson University
Generating an episomally maintained transgene vector in *Naegleria fowleri*

P21 **Aidan May**, Center for Tropical and Global Diseases, UGA
Utilizing the ATP FRET sensor ATeam3.10 to quantify mitochondrial ATP concentration changes in *Toxoplasma gondii*

P22 **Samuel Nyarko**, CTEGD & Department of Cellular Biology, UGA
Exploring dedaquiline as an apicomplexan ATP synthase inhibitor

P23 **Anthony Ruberto**, Center for Tropical and Emerging Global Diseases UGA
Lead optimization and target identification of a new series of antimalarial compounds targeting *Plasmodium vivax* hypnozoites: opportunities and challenges

P24 **Zhe Cheng**, CTEGD & Department of Cellular Biology, UGA
Discovery and characterization of overlapping chromosome 10 copy number variance in multiple in vitro selected artemisinin resistant *Plasmodium falciparum*

P25 **Nathan Chasen**, Department of Cellular Biology UGA
Nested genes of apicomplexan parasites and a potential 'Tag-in-Place' strategy

P26 **Wayne Cheng**, CTEGD & Center for Vaccines and Immunology, CVM, UGA
Increased Duffy binding protein 1 expression correlates with *Plasmodium cynomolgi* growth in continuous culture

P27 **Magdalena Argomaniz**, CTEGD & Center for Vaccines and Immunology, CVM, UGA
A *Plasmodium vivax* strain that expresses fluorescent proteins throughout the life cycle

P28 **Hannah Abbey**, EPIC & Department of Genetics and Biochemistry, Clemson University
Resolving the function of the SET domain protein lysine methyltransferase in *Trypanosoma brucei*

P29 **Katherine Moen**, CTEGD & Department of Cellular Biology, UGA
Redox regulation of calcium homeostasis in *Toxoplasma gondii* for optimal lytic cycle progression

P30 **Mackenzie Sievert**, Center for Tropical and Emerging Global Diseases, UGA
Comprehensive QTL mapping in a Kelch13 wildtype *Plasmodium falciparum* genetic cross

P31 **Ganesh Babu Malli Mohan**, Center for Tropical and Emerging Global Diseases, UGA
Stage-specific and temperature-responsive control of protein stability in *Trypanosoma cruzi* using a DHFR destabilizing domain system

P32 **Melissa Sleda**, Center for Tropical and Emerging Global Diseases, UGA
Two historical 4(1H)-quinolone scaffolds have potent efficacy against acute and chronic stages of *Toxoplasma gondii*

P33 **Benjamin Phipps**, Department of Genetics UGA
Additional blood meals after infection increase fitness of malaria parasites and their mosquito host

P34 **Chandler Lowe**, CTEGD & Department of Genetics, UGA
Investigating Notch signaling in *Hymenolepis diminuta* segmentation

P35 **James Oristian**, CTEGD & Department of Infectious Diseases, UGA
Induced in vitro sexual commitment of *Plasmodium cynomolgi*

P36 **Aylla von Ermland**, Center for Tropical and Emerging Global Diseases, UGA
Investigating antigen diversification of *Trypanosoma cruzi* within a single-host infection

P37 **Lyric Wardlaw**, Department of Biochemistry and Molecular Biology, UGA
Identification of β-carboline derivatives active against quiescent artemisinin-resistant *Plasmodium falciparum*

P38 **Cierra Gladfelter**, CTEGD and Dept. of Genetics, UGA
Understanding the role of *nanos* in germ cell development and regeneration in *Hymenolepis diminuta*

P39 **Jose Saenz**, Center for Tropical and Emerging Global Diseases, UGA
Understanding how *T. cruzi* infection is controlled in muscle

P40 **Melissa Rogers**, CTEGD & Department of Cellular Biology, UGA
Investigating the role of putative membrane contact site proteins in *Toxoplasma gondii*

P41 **Nupur Kittur**, Center for Tropical and Emerging Global Diseases, UGA
VEuPathDB: Tools for genomic-scale data exploration, analysis, integration and discovery

P42 **Abdul Malik Hussein**, CTEGD & Department of Cellular Biology, UGA
Membrane contact site assembly is required for VDAC-dependent mitochondrial calcium uptake In *Toxoplasma gondii*

P43 **Ruby Harrison**, Center for Tropical and Emerging Global Diseases, UGA
Preliminary characterization of two *Trypanosoma cruz*i isolates from northern Florida, U.S., suggests the potential for human infection

P44 **Gaurav Kumar**, Department of Molecular and Cellular Biology, Kennesaw State Universit
Tb927.8.2820, a target of NEU-4438, is important for endocytosis of transferrin and cell shape maintenance in *Trypanosoma brucei*

P45 **Grace Vick**, CTEGD & Department of Infectious Diseases, UGA
A SNARE-like *Plasmodium* rhoptry neck protein is required for sealing of the parasitophorous vacuole during merozoite invasion

P46 **Katie Dillon**, Institute of Bioinformatics UGA
Tick Genomes: Overcoming the limitations of tick biology with advancements in sequencing technology

P47 **Amadis Vivas**, CTEGD & CVI, UGA
Immunogenicity of a protein nanoparticle vaccine encoding the *Plasmodium falciparum* MIF protein in *Aotus nancymaae*

P48 **Caroline Palmentiero**, EPIC & Department of Genetics and Biochemistry, Clemson University
Establishment of transfection approaches in *Naegleria fowleri*

P49 **Jose Maravi-Jaime**, Universidad Peruana Cayetano Heredia, Lima, Peru
Differentially expressed genes in the in vitro activation of *Taenia solium* larvae by taurocholic acid

Plasmodium spp. differential susceptibility to antimalarials targeting parasite mitochondria during the vector stages

Justine C. Shiau[1,2,3], Mastura Ruma[1,2], Chester J. Joyner[1,2,4], Douglas G. Paton[1,2,3,4,5]
[1]Department of Infectious Diseases, [2]Center of Tropical & Emerging Global Diseases, [3]Precision One Health, and [4]Center for Vaccine & Immunology, Athens GA [5]Savanah River Ecology Laboratory, Akin, SC

Plasmodium parasites are the causative agent of malaria, responsible for over 2 million cases yearly. While *P. falciparum* is the most abundant and virulent species, *P. vivax* is geographical more widespread and possesses a unique dormancy stage in the human liver that presents challenges to disease elimination and control. Along with colleagues, we have proposed a novel transmission control approach based on the exposure of vector mosquitoes to antimalarial drugs to achieve malaria transmission blockade. Towards this, we have demonstrated that brief mosquito contact with the antimalarial atovaquone (ATQ) can prevent transmission of *P. falciparum* to mosquitoes, and thus onward transmission to subsequent huma hosts. Given the importance of targeting non-*falciparum Plasmodium* species for the elimination of malaria, we set out to test the efficacy of this approach against both *P. vivax* and the closely related primate malaria parasite *P. cynomolgi*. *Anopheles dirus* mosquitoes were exposed to ATQ and the experimental mitochondrial inhibitors ELQ613 and ELQ453 both before and after mosquito acquisition of *Plasmodium* infection, and through different drug delivery routes. Interestingly, we observed significant divergence in susceptibility between *P. falciparum* and *P. cynomolgi,* with the latter parasite exhibiting significantly lower susceptibility to inhibition by all three tested compounds, especially ATQ. This observed differential susceptibility may suggest that ATQ, a commonly used drug for travelers prophylaxis, may be less effective than previously thought against *vivax*-group parasites.

Exploration of putative sodium/proton exchangers in *Trypanosoma brucei*

Sabrina Pizarro[1,2], Daniel Call[3], Ken Christensen[3], James Morris[1,2]
[1]Clemson University Eukaryotic Pathogens Innovation Center, [2]Clemson University Department of Genetics and Biochemistry, [3]Brigham Young University Department of Chemistry and Biochemistry

Trypanosoma brucei, the causative agent of African sleeping sickness, remains a medical and agricultural concern for much of Sub-Saharan Africa. Glycolysis is critical to the infectious blood stream form parasite, and multiple glycolytic enzymes, some of which are regulated by pH, have been validated as potential drug targets. Two putative sodium proton exchangers (NHE1 and NHE2) were identified in the *Trypanosoma brucei* genome, and we hypothesize they play a role in the regulation of glycosomal pH. RNAi constructs targeting the two NHE proteins were transfected into lines expressing either a pH or glucose biosensor allowing us to monitor the pH and glucose fluctuations when these proteins are down regulated. Endogenously tagged NHE lines are being used to identify subcellular localization and changes in expression resulting from environmental manipulations. Further understanding of glycosomal regulation will aid in the identification of novel drug targets.

Memory regulatory T cells protect against recurrent malaria

Nana Charles-Chess[1,2]; Samarchith Kurup[1,2]
[1]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, Georgia, USA; [2]Department of Cellular Biology, University of Georgia, Athens, Georgia, USA

Regulatory T cells (Tregs) are a subset of CD4 'helper' T cells known for their role in restraining pro-inflammatory immune responses and impeding the effective control of *Plasmodium* parasites during primary blood-stage malaria. Following primary infection, Tregs persist and form a stable memory Treg (mTreg) population. However, the role of mTregs in recurrent malaria remains unknown. Given that individuals living in malaria-endemic regions experience repeated infections with *Plasmodium*, it is crucial to understand how mTregs influence immunity during recurrent infections. Relying on longitudinal studies in both humans and mice, we show that mTregs generated following *Plasmodium* infection acquire protective functions when recalled. Notably, the *Plasmodium*-experienced mTregs undergo antigen-driven expansion and inflammation-induced epigenetic reprogramming to transition from FOXP3$^+$ immunosuppressive cells to Bcl6$^+$ follicular T helper (Tfh)-like effectors, which enhance germinal center responses and the generation of *Plasmodium*-specific antibodies, ultimately facilitating malaria control. Preventing such Tfh differentiation abolished protection. Our findings reveal a previously unrecognized context-dependent adaptive plasticity in mTregs that enables their functional switch from immunoregulatory to protective effectors during recurrent infections, revealing new avenues to harness Treg plasticity in vaccines or immunotherapies.

Humoral immunity leads to control of chronic *Plasmodium* infections

Saniya S. Sabnis[1,2,3], Celia Saney[2], Monica Cabrera-Mora[4], the MaHPIC Consortium[4], Regina Joice-Cordy[5], Alberto Moreno[4,6], Ignacio Sanz[6], F. Eun-Hyung Lee[6] Tracey J. Lamb[7], Mary R. Galinski[4,6], Chester J. Joyner[1,2,3]
[1]Dept. of Infectious Diseases, UGA, Athens, GA [2]CVI, UGA, Athens, GA [3]CTEGD, UGA, Athens, GA [4]Yerkes, Emory, Atlanta, GA [5]Dept. of Biology, Winston-Salem, NC [6]Dept. of Medicine, Emory, Atlanta, GA [7]Dept. of Pathology, UU, Salt Lake City, UT

Chronic, minimally symptomatic *Plasmodium falciparum* (*Pf*) infections are common in endemic areas. These infections predispose individuals to secondary bacterial infections and may reduce malaria vaccine efficacy. Thus, determining how chronic infections develop and the immunological changes that occur is essential for ameliorating disease and developing new interventions. Here, we used samples collected from rhesus macaques infected with *P. coatneyi* (*Pco*), a model of *Pf* malaria, to determine the host responses leading to control and establishing a chronic infection. Based on whole blood transcriptomics, infections reached chronicity 50-80 days after sporozoite infection. B cell pathways are upregulated during chronic infections and are correlated with parasite control and reduced symptoms. IgG and IgM antibodies against the neutralizing epitope MSP-1_{19} peak when parasitemia is controlled and remain elevated. Interestingly, the magnitude of the MSP-1_{19} IgM antibody response delineated when each animal would control parasitemia and not MSP-1_{19} IgG. In agreement, antibodies inhibited parasite growth *in vitro* and predicted when animals would control parasitemia. Together, this study defines the development of chronic *Plasmodium* infections and demonstrates that humoral immune response is key to establishing chronicity.

Same difference: An apicomplexan-specific extension in ATP synthase subunit f in *Toxoplasma gondii*

Kaelynn Parker[1,3] and Diego Huet[2,3]

[1]Department of Cellular Biology, University of Georgia, Athens 30602, GA, USA [2]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens 30602, GA, USA [3]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens 30602, GA, USA. diego.huet@uga.edu

The mitochondrion is considered the powerhouse of the cell because it houses the mitochondrial ATP synthase, an enzyme capable of harvesting the proton gradient generated by the electron transport chain to generate ATP. *Toxoplasma gondii*, a member of the parasitic phylum Apicomplexa, possesses a considerably larger ATP synthase than common model organisms, with 17 apicomplexan-specific subunits and phylum-specific extensions on several of its canonical subunits. One with such an extension is subunit f (TgATPf), which contains an apicomplexan-specific extension. Unexpectedly, a thermal proteome profiling study in *T. gondii* found subunit f to be the most responsive protein to calcium. More specifically, it is destabilized by calcium. In yeast and mammals, a transient spike of calcium in the mitochondrial matrix results in an increase in ATP production via oxidative phosphorylation. However, direct calcium interactions with the ATP synthase have not yet been implicated in the regulation of ATP production. Here, I have shown that TgATPf is essential for parasite lifecycle, cristae density formation, and mitochondrial ATP production. Future work will investigate the potential interaction of TgATPf with calcium, the regulation of the *T. gondii* ATP synthase by this ion, and its role in the lytic cycle using a novel inducible complementation system. Results of this work will elucidate the function of this apicomplexan specific extension in TgATPf and potentially uncover a parasite-specific mechanism of ATP synthase regulation through calcium interactions.

Infection in the Collaborative Cross reveals differential susceptibility and resistance to *N. fowleri*

Joseph Dainis[1,2], Perla Vazquez[1], Anne Elliot[1], Dennis Kyle[1,2,3]

[1]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, Georgia, USA; [2]Department of Infectious Diseases, University of Georgia, Athens, Georgia, USA; [3]Department of Cellular Biology, University of Georgia, Athens, Georgia USA

Naegleria fowleri (Nf), also known as the brain-eating amoeba, is a eukaryotic, free-living species of amoeba that is the causative agent of Primary Amoebic Meningoencephalitis (PAM). Infection occurs when amoebae enter the nasal passages in contaminated water and migrate towards the brain, where they feed on neural tissue. *Nf* has a very peculiar infection pattern; many successful infectious agents, such as COVID-19 or *Plasmodium* spp., have millions of new cases each year, with mortality rates averaging less than 1%. In comparison, only 5 PAM cases on average are documented in the U.S. each year, with a severe 97% mortality rate in infected individuals. Surveillance efforts suggest that *Nf* lives ubiquitously in freshwater across the globe, putting millions of individuals at risk every year for PAM. How is it that *Nf* has this highly unusual infection pattern with such dire disease outcomes in humans? We hypothesize that genetic differences in key host factors correlate with susceptibility to *Nf* infection. To test our hypothesis, we have infected 30 mouse lines from the Collaborative Cross (CC) mouse series, a model of genetic diversity that represents over 90% of the common genetic variations found in laboratory mice, with 5,000 lowly virulent *Nf* amoebae. Infections in these mice reveal highly diverse disease outcomes. Some CC lines are highly susceptible, manifesting in end-stage disease 3-5 days faster than control mice. Other CC lines are able to resist the infection, with a majority of the mice surviving. Initial results support the role of genetic diversity in disease outcomes with *Nf*, and future studies will look to identify specific genetic determinants controlling susceptibility and resistance phenotypes.

Field-isolated bacteria support larval mosquito development under nutrient-limited conditions

Derek T. Huck[1,2], Xiushuai Yang[1], Samantha Denny[3], Michael R. Strand[1,2]
[1]Department of Entomology, University of Georgia, Athens, Georgia, USA; [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, USA; [3]Department of Physiology and Pharmacology, University of Georgia, Athens, GA, USA

Mosquitoes like *Aedes aegypti* are best known as blood-feeding vectors of diseases including dengue, yellow fever, and filariasis. Pathogen transmission crucially depends on the abundance of adults in a given population that are competent vectors. Adult abundance is determined by the development of larval mosquitoes that are strictly aquatic. One factor that substantially influences larval development is resource acquisition. Field habitats where mosquito larvae develop primarily contain plant detritus that forms the base of the food web and microbial communities which consist of one or more trophic levels. Mosquito larvae are usually the top-level consumers but the roles of detritus and microbes as resources for development into adults are largely unclear. In this study, we used both field-collected plant detritus and an artificial nutritionally-limited diet to isolate and identify microbes that facilitate the development of *A. aegypti* under nutrient-poor conditions. Experiments using recently-developed methods for producing axenic mosquito cultures with no microbes found that axenic larvae fail to develop on both plant detritus and our artificial diet. However, larval development was rescued by adding cultures of undefined microbial communities obtained from field sites. Furthermore, we identified simplified communities of 4-8 bacterial species that supported development of larvae into adults on detritus diets as well as a single bacterial species that supported development on our artificial diet. Overall, our results suggest that while the presence of microbes that provision nutrients deficient in the diet are essential for mosquito development, the identities of these microbes differ depending on nutritional conditions.

Exploring the genome of a global pathogen: New insights into *Cryptosporidium parvum*

Agyabeng-Dadzie, F.[1*], Baptista R. P.[2], Kissinger J. C.[1,3,4], Glenn T. C.[1,3,5]
[1]Department of Genetics, [3]Institute of Bioinformatics, [4]Center for Tropical and Emerging Global Diseases, and [5]Dept. of Environmental Health Science, University of Georgia, Athens, GA, USA; [2]Houston Methodist Research Institute, Houston, TX, USA

Cryptosporidium is the second leading cause of diarrheal disease worldwide, with particularly severe effects in infants under two in low- and middle-income countries and immunocompromised individuals. Despite its health importance, genomic data remain limited and skewed; approximately 50% of genome assemblies represent only two species, *C. hominis* and *C. parvum* (1), while other key species are underrepresented. Most genome assemblies are highly fragmented due to reliance on short-reads, and only *C. parvum* has a complete telomere-to-telomere genome sequence (CpBGF T2T) (2). We analyzed 29 *C. parvum* genome sequences and identified 2,511 single-copy genes in ≥95% of assemblies, including highly variable genes like Mucin (*cgd3_720*). Using 548 *C. parvum* SRA datasets downloaded from GenBank, we conducted the first large-scale whole-genome single-nucleotide polymorphism analysis of *C. parvum*. We observe a significant regional bias—68% of usable samples were from the U.S., China, or the U.K., underscoring the need for more diverse sampling. Our analysis revealed that most samples could be classified into distinct populations, but some have evidence of mixed ancestry. Our framework allows accurate sample categorization, detects mixed infections, and supports genotyping efforts like CryptoCapture. Conserved single-copy genes offer a reliable alternative for population studies without full genome assemblies, helping to track transmission and understand evolutionary trends. **References:** doi: 10.1007/s40475-024-00318-y[1], doi: 10.1101/2023.06.13.544219[2]

Unraveling the enigmatic feeding apparatus of *Trypanosoma cruzi*

Gonzalo Seminario-Mondejar[1], Ronald Etheridge[1]
[1]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens

Among the pathogenic trypanosomatids, *Trypanosoma cruzi* alone retains the ancestral cyto**s**tome-cyto**p**harynx **c**omplex (SPC) feeding apparatus, a structure which it shares with its free-living bacterivorous kinetoplastid relatives. Despite having a crucial role in nutrient acquisition, our understanding of the SPC's construction and molecular mechanics remains extremely limited. Previously, our lab utilized a combination of bioinformatic and co-immunoprecipitation methods to identify SPC-localized proteins crucial for endocytosis. However, the inherent limitations of these approaches mean we still lack a detailed picture of the full molecular complexity of this organelle. To expand our knowledge of the proteomic composition of the SPC, we have adapted the promiscuous biotin ligase TurboID for use in *T. cruzi*. We fused TurboID to the myosin-associated protein (MyAP), previously identified as essential for endocytosis. Proximity labeling, followed by mass spectrometry analysis, identified over 100 high-confidence MyAP-proximal proteins, yielding novel insights into the structural and regulatory components of the SPC's rootlet microtubules. Encouragingly, most newly identified proteins lacked orthologs in the SPC-deficient *T. brucei*, yet are conserved in SPC-bearing free-living kinetoplastids. Using our in-house conditional knock-down and tagging system, we verified SPC localization for the highest-ranked candidates and assessed their role in endocytosis. Importantly, we have identified 2 kinesin motors which we show play critical roles in SPC-mediated endocytosis, thus revealing an unrecognized kinesin-driven aspect of this organelle's function. These findings provide the first comprehensive molecular portrait of SPC rootlet fibers in any protozoan and uncover critical kinesin components, advancing our understanding of this unique and enigmatic endocytic apparatus.

A putative glycosyltransferase is required for *Plasmodium falciparum* asexual development

Alejandra Villegas Lopez and Vasant Muralidharan
University of Georgia

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.

Poster Presentations

P1. Resistance unraveled: Dissecting drug sensitivity and resistance mechanisms in *Cryptococcus neoformans*

Toya Tanner, Blake Billmyre
University of Georgia, Athens, GA

Cryptococcus neoformans is a human fungal pathogen responsible for approximately 147,000 deaths annually, predominantly affecting immunocompromised individuals. Current antifungal therapies, including azoles like fluconazole, are limited by toxicity and rising resistance, necessitating the identification of novel drug targets and mechanisms of action. Using a TN-Seq-based approach, we examined the genetic determinants underlying drug sensitivity and resistance in *C. neoformans*. TN-Seq is a high-throughput method used to identify genes linked to specific traits. Here, we examined four mutants of genes predicted to exhibit differential sensitivity to fluconazole. We tested them for resistance phenotypes to fluconazole, fenpropimorph, carboxin, and difenoconazole. All the mutants responded to fluconazole as predicted by TN-Seq. However, some showed resistance to one drug while remaining sensitive to others. These results suggest that the mechanisms underlying drug sensitivity and resistance may differ between compounds, even those targeting similar pathways. To further investigate these differences, we are now assessing how these mutants respond to oxidative, osmotic, and cell wall stressors. We also plan on testing the hypothesis that changes in efflux pump activity and gene expression under fluconazole exposure explain the difference in drug resistance in mutants with consistent phenotypes across multiple drugs. This work aims to uncover genes and pathways that influence drug response, providing insights into potential therapeutic interventions. Future work will focus on further characterizing these mutants and optimizing experimental methods to better understand antifungal resistance.

P2. Enhanced neutralizing antibodies and protection against RSV by new pre-fusion mRNA and subunit protein combination vaccines in mice

Surya Sekhar Pal, Ki-Hye Kim, Noopur Bhatnagar, Rong Liu, Jannatul Ruhan Raha, Bo Ryoung Park, Chong-Hyun Shin, Sang-Moo Kang
Center for Inflammation, Immunity and Infection, Institute for Biomedical Science, Georgia State University, Atlanta, Georgia

Human respiratory syncytial virus (RSV) causes high hospitalization and mortality in children and elderly. RSV pre-fusion stabilized protein vaccines have been licensed for elderly and maternal vaccination. Nonetheless, there exists an urgent demand for a safer and effective RSV vaccine in elderly and young children, avoiding vaccine-enhanced disease. We developed a new pre-fusion mRNA construct (F1d-dcmTM) containing additional mutations as well as prototype pre-fusion stabilizing mutations (DS-Cav1). New pre-fusion F1d-dcmTM mRNA encapsulated in lipid nanoparticles (LNP) was found to be more effective in inducing neutralizing antibodies after vaccination of mice than prototype DS-Cav1 Pre-fusion protein. Remarkably, we found that combined F1d-dcmTM mRNA-LNP and Pre-fusion protein vaccination elicited significantly higher amounts of RSV neutralizing antibodies in mice than those by either vaccine alone. After challenge of vaccinated mice with RSV, F1d-dcmTM mRNA-LNP and combination group with pre-fusion protein provided effective protection by clearing lung viral loads, preventing lung histopathology and inflammation, and inducing balanced T cell responses, compared to those by prototype pre-fusion protein. These new RSV pre-fusion mRNA vaccine construct and a strategy of combined vaccination would provide safer and more effective protection than current prototype RSV pre-fusion subunit vaccine.

P3. Enolase inhibitors as therapeutic agents for *Naegleria fowleri* infection

Jillian E. M. McKeon[1], Samuel Kwain[2], Caroline Palmentiero[1], Colm P. Roster[1], Rooksana E. Noorai[3], Dan C. Whitehead[2], James C. Morris[1]

Eukaryotic Pathogens Innovation Center, [1]Department of Genetics and Biochemistry, [2]Department of Chemistry, [3]Clemson University Genomics and Bioinformatics Facility, Clemson University, Clemson, SC

Current treatments for *Naegleria fowleri* infections are inefficacious and mortality rates remain over 95%. We have previously demonstrated the importance of glycolysis to the amoebae (Milanes, 2018), suggesting inhibitors of glycolytic enzymes could be potent therapeutic agents. Supporting this postulate, we have found that human enolase 2 (ENO2) phosphonate inhibitors are potent inhibitors of recombinant *Nf*ENO, with the most potent, (1-hydroxy-2-oxopiperidin-3-yl) phosphonic acid (HEX) having an IC_{50} value of 0.14 ± 0.04 µM. HEX was also a potent amebicide, with an EC_{50} value of 0.21 ± 0.02 µM while the CC_{50} value was >300 µM (Milanes, 2024). Gradual exposure to the compound in culture over several months generated slow growing amoebae with EC_{50} values 10-fold higher than parental lines. RNA sequencing of the resistant line revealed upregulation of transcripts involved in metabolic processes, including glucose 6-phosphate isomerase and fructose-bisphosphate aldolase, genes upstream of enolase. To assess the potential of HEX as a monotherapy for amoebae infection, infected rodents were treated by intranasal HEX instillation. This treatment increased longevity, with eight of 12 HEX-treated rodents surviving the experimental period (resulting in an indeterminable median survival time). Only 1 of 12 vehicle-treated rodents remained at the end of the experimental period, yielding a median survival time of 10.9 days. Brain extraction of the surviving infected animals revealed that six of the eight survivors remained infected, indicating that HEX suppressed the infection but did not eliminate it (Milanes, 2024). To assess the potential of HEX as a partner therapeutic, we have assessed synergy in combination with amphotericin B and miltefosine, with results suggesting an additive effect of the agents. In summary, the phosphonate based ENO2 inhibitors are potent *Nf*ENO inhibitors, toxic to *Naegleria* in culture, and have promising activity in a rodent model of disease, suggesting these compounds could be further developed for use in treatment of infections either alone or in combination with other therapies.

P4. Modulation of host cell apoptosis by secreted effectors during *Toxoplasma gondii* infection

Kenna Berg[1,2] and Alex Rosenberg[1, 2]

[1]CTEGD, UGA, Athens, GA [2]Dept. of Infectious Diseases, CVM, UGA, Athens, GA

To effectively modulate the host cell response following invasion, the intracellular parasite *Toxoplasma gondii* secretes a suite of effector proteins into the host cell via the MYR1 translocon complex. These effectors help regulate inflammatory signaling pathways, including NF-κB activation. Deletion of the MYR1 complex—and the resulting block in effector secretion—significantly impairs the parasite's ability to control host immune responses. In addition to immune modulation, *T. gondii* is known to inhibit host cell apoptosis, although the underlying mechanisms have remained largely unclear. In this study, we show that knockout of the MYR1 complex compromises the parasite's ability to suppress host cell apoptosis, likely through disruption of NF-κB signaling. Our findings reveal a functional connection between MYR1-secreted effectors and the parasite's capacity to block apoptotic cell death, offering new insight into the molecular strategies *T. gondii* uses to manipulate host cell fate.

P5. Essential roles of vacuolar transporter chaperones 1 and 4 in polyphosphate metabolism and *T. cruzi* infectivity

Mayara S. Bertolini[1], Miguel A. Chiurillo[1], and Roberto Docampo[1]
[1]Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Georgia, United States

Polyphosphate (polyP) is a polymer of inorganic phosphate that typically accumulates in acidic, calcium-rich organelles known as acidocalcisomes. The synthesis of polyP in eukaryotes was not well understood until it was demonstrated that the vacuolar transporter chaperone (VTC) complex in *Saccharomyces cerevisiae*, composed of Vtc1-Vtc5, functions as a polyP polymerase that synthesizes polyP from adenosine triphosphate (ATP) and translocate it across the vacuolar membrane. The human pathogen *Trypanosoma cruzi*, which causes Chagas disease, possesses homologs of Vtc1 (TcVtc1) and Vtc4 (TcVtc4). It has been shown that TcVtc4 catalyzes polyP synthesis and localizes to acidocalcisomes in this parasite. Here, we report the use of a CRISPR/Cas9-based strategy to generate knockout mutants of *TcVtc1* and *TcVtc4*. We successfully obtained *TcVtc1*-KO parasites, and after several attempts, we confirmed by PCR and Southern blot analyses that only one *TcVtc4* allele was replaced by the DNA donor cassette at the specific locus. This result suggests that null alleles may have lethal effects in epimastigotes. Both *TcVtc1*-KO and *TcVtc4*-SKO parasites exhibit a lower proliferation rate and are defective in short-chain polyP synthesis compared to control cells. Moreover, *TcVtc1*-KO and *TcVtc4*-SKO trypomastigotes display a reduced capacity to invade host cells and replicate within them as amastigotes. Since *TcVtc1* and *TcVtc4* are crucial in epimastigotes and critical for the infective stages, knockdown cell lines are also being generated to further investigate their roles in *T. cruzi*. Given that *VTC* genes are absent in the genomes of higher eukaryotes, drugs targeting TcVtc1 and TcVtc4 function may hold promise as therapeutic candidates for Chagas disease.

P6. Stereospecific resistance to N2-acyl tetrahydro-β-carboline antimalarials is mediated by a PfMDR1 mutation that confers collateral drug sensitivity

Emily K. Bremers[1,2], Joshua H. Butler[1,2], Leticia Do Amaral[1,2], Emilio F. Merino[1,2], Hanan Almolhim[3], Bo Zhou[4], Rodrigo P. Baptista[2], Maxim Totrov[6], Paul R. Carlier[3,4], and Maria B. Cassera[1,2]
[1]Department of Biochemistry and Molecular Biology, University of Georgia, [2]Center for Tropical and Emerging Global Diseases, University of Georgia, [3]Department of Chemistry, Virginia Tech, [4]Department of Pharmaceutical Sciences, University of Illinois Chicago, [5]MolSoft LLC, San Diego, California

Half the world's population is at risk of developing a malaria infection, which is caused by parasites of the genus *Plasmodium.* Currently, resistance has been identified to all clinically available antimalarials, highlighting an urgent need to develop novel treatments and better understand common mechanisms of resistance. We have previously identified a novel tetrahydro-β-carboline compound, PRC1590, which potently kills the malaria parasite. To better understand its mechanism of action, we selected for and characterized resistance to PRC1590 in *P. falciparum*. Through *in vitro* selection of resistance to PRC1590, we identified that a single nucleotide polymorphism on the parasite's multidrug resistance protein 1 (PfMDR1 G293V) mediates resistance to PRC1590. This mutation results in stereospecific resistance and sensitizes parasites to other antimalarials such as mefloquine and MMV019017. Stage specificity assays revealed that PRC1590 is most potent in the trophozoite stage, when the parasite forms a single digestive vacuole (DV). Moreover, fluorescence microscopy revealed that PRC1590 disrupts DV function, indicating a potential molecular target associated with this organelle. Our findings mark a significant step in understanding the mechanism of resistance and the mode of action of this emerging class of antimalarials. Our results also suggest a potential link between PfMDR1 resistance and molecular target.

P7. Conditioned media from differently virulent *Naegleria fowleri* differentially induces cytopathic effects over mammalian cell lines

Perla Vazquez[1], Joseph Dainis[1,2], Dennis Kyle[1,2,3]
[1]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, Georgia, USA; [2]Department of Infectious Diseases, University of Georgia, Athens, Georgia, USA; [3]Department of Cellular Biology, University of Georgia, Athens, Georgia USA

Naegleria fowleri (*Nf*), also known as the "brain-eating" amoeba, is a eukaryotic, free-living amoeba that is the causative agent of primary amoebic meningoencephalitis (PAM) that holds a >97% mortality rate. Prior studies in the lab have demonstrated that different clinical isolates of *Nf* are differentially virulent, with varied disease outcomes in infected mice and distinct feeding rates over mammalian cells. Secreted factors present in CM from infectious agents, such as *Entamoeba histolytica*, have been shown to mediate virulence over target host cells; however, this has not yet been well-defined for *Nf*. Previously, it has been shown that conditioned media (CM) from passaged amoebae induces more rapid cell clearance when co-incubated with axenic amoebae. The aim of this work is to characterize this established phenotype with differentially virulent *Nf* isolates and determine whether CM mediates virulence over specific host cells. We found that 3 different isolates exposed to differentially virulent *Nf* CM exhibit rates of cell clearance proportional to the intrinsic virulence level of the CM. We also conducted these assays with three mammalian cell lines – Vero, HFF, and B103 cells – and observed differences in the cytopathic effects mediated by virulent *Nf* CM. This suggests that particular cell types are selectively affected by the *Nf* secretome and that *Nf* can preferentially induce faster feeding over these mammalian cells through its secretome. Future directions will elucidate components of the *Nf* secretome and mammalian cells driving these phenotypes.

P8. A *Plasmodium* transmembrane protein is essential for asexual segmentation of *Plasmodium falciparum*

Watcharatip Dedkhad, Manuel Fierro, Tia Tran, Vasant Muralidharan
University of Georgia

Malaria is a life-threatening disease caused by parasites in the genus *Plasmodium*. Clinical manifestations of the disease are caused by a successful asexual replication in the red blood cells (RBC) known as schizogony. The *Plasmodium* schizogony is the process that produces multiple nuclei in one cytoplasm, followed by a final segmentation to generate individual invasive merozoites. We show that a putative transmembrane protein, Pf3D7_1308000 or Pf4TM, is essential for successful sexual segmentation. We generated mutants for Pf4TM using rapamycin (RAP)-inducible DiCre recombinase knockout system. Using immunofluorescence microscopy, we localize Pf4TM to vesicles that do not co-localize with any known vesicular markers. We showed that treating Pf4TM^{KO} with RAP inhibits parasite growth, suggesting that Pf4TM is essential for the blood stage life cycle. Giemsa-stained thin blood smears of Pf4TM^{KO} parasites showed that DMSO-treated Pf4TM parasites could egress, reinvade fresh RBCs, and develop into ring-stage parasites. In contrast, we found that RAP-treated Pf4TM^{KO} developed from a ring to multi-nucleated schizonts that failed to form new rings in the next asexual cycle. Live imaging of RAP-treated Pf4TM^{KO} parasites showed that parasites initiate egress, but merozoites were attached to residual bodies. Next, we utilized ultrastructure expansion microscopy (U-ExM) to determine the architecture of late-stage segmented schizonts and found that RAP-treated Pf4TM^{KO} parasites presented abnormal plasma membranes, indicated by anti-MSP1 and anti-AMA1 staining. We hypothesize that Pf4TM is essential for asexual segmentation. Next, we will determine the localization and specific role of Pf4TM in merozoite segmentation during schizogony.

P9. Mechanism of antigen-presenting cell recruitment during liver-stage malaria

Clyde Schmidt-Silva[1], Samarchith Kurup[1]
[1]University of Georgia

Plasmodium parasites have to undergo development in the liver before progressing to the potentially lethal blood stage of malaria. Therefore, the liver stage of malaria represents an ideal target for vaccine development. Vaccination with attenuated *Plasmodium* sporozoites, such as radiation-attenuated sporozoites (RAS), induces robust CD8 T cell responses that target and clear the infected hepatocytes during subsequent infections, impeding the onset of blood-stage malaria. RAS establish abortive infections in hepatocytes, triggering pyroptotic cell death and release of *Plasmodium* antigens into the extracellular environment of the liver. $CSF1R^+$ antigen-presenting cells (APCs) recruited to the liver capture these antigens and prime CD8 T cell response in the liver-draining lymph nodes. The strength of $CSF1R^+$ APC recruitment is a key determinant of the magnitude of CD8 T cell responses and RAS-mediated protection against malaria. However, the mechanism of such APC recruitment remains unknown. We show that the liver-resident macrophages, Kupffer cells (KC), acquire inflammasome complexes released from pyroptotic hepatocytes and drive adoptive-inflammasome activation (AIA), generating mature IL-1. This IL-1 in turn induces CCL2 and CCL7 in specific liver non-parenchymal cells, driving CCR2-mediated recruitment of $CSF1R^+$ APC to the liver from circulation. In addition to advancing our current understanding of immune cell dynamics in the liver, we expect this work to inform the generation of more immunogenic malaria vaccines.

P10. Parasite-resistant sheep exhibit metabolic efficiency after a *Haemonchus contortus* priming infection

H.G. Teddleton[1], A.D. Wise[2], A.J. Monteith[2], and E. Shepherd[1]
[1]Department of Animal Science, University of Tennessee, Knoxville, [2]Department of Microbiology, University of Tennessee, Knoxville

Grazing ruminants are subject to pathologies from gastrointestinal nematode *Haemonchus contortus,* where infection in young or susceptible animals can cause extreme anemia and even result in death. Parasite-resistant St. Croix sheep (STC) generate strong T helper type-2 (TH2) responses resulting in a rapid clearance of *H. contortus*, while parasite-susceptible Suffolk sheep (SUF) have delayed responses. With this, metabolism within immune cells can alter the microenvironment which influences the hosts ability to respond to pathogens. However, metabolism of immune cell populations in SUF and STC is not known, nor how this relationship can impact overall host responses against helminth infections. Thus, the objective of this study was to measure mitochondrial respiration in context of helminth infection utilizing a model of parasite susceptibility and resistance in sheep. To address this, peripheral blood mononuclear cells (PBMCs) were isolated from parasite naive, infected, and primed SUF and STC to measure mitochondrial respiration and mitochondrial biomass. Oxygen consumption rates (OCR) and extracellular acidification (ECAR) rates were determined via a Seahorse XFe96 analyzer and analyzed using Wave Desktop software (Agilent). Both naive and primed STC-derived PBMCs expressed an increase of oxidative phosphorylation across all metrics, when compared to naive and primed SUF ($P<0.05$). After 28 days of *H. contortus* infection, STC indicated a metabolic shift to glycolysis, yet no differences were observed in OCR at d28 in SUF-derived PMC ($P<0.05$). Interestingly, primed STC had decreased mitochondrial biomass, yet increased ATP production when compared to SUF, indicating differences in metabolic profiles between parasite-resistant and susceptible sheep.

P11. Uncovering the potential role of dsRNAs in *Cryptosporidium* gene regulation

Samantha Gunasekera[1], Rui Xiao[2], Jessica Kissinger[1,3,4]
[1]CTEGD, UGA, Athens, GA, [2]CHMI, Dept. of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, [3]Dept. of Genetics, UGA, Athens, GA, [4]Institute of Bioinformatics, UGA, Athens, GA

Cryptosporidium is an enteric protozoan parasite and a major contributor to diarrhea-associated morbidity and mortality in children under two years of age. *Cryptosporidium* is entirely dependent on its host to salvage nucleotides from for DNA replication and transcription and has evolved to have a highly compact genome of ~9.1 M spanning eight chromosomes with ~4,850 genes, most of which overlap in the untranslated regions. Prior work within the research group has demonstrated that many of these genes encode coding and non-coding antisense transcripts. *Cryptosporidium* is the most basal branching member of the Apicomplexa phylum and while the transcriptional regulatory networks in this genus remain incompletely understood, existing evidence provides many clues that gene regulation in *Cryptosporidium* may diverge from the broader Apicomplexa model. Regions of overlapping antisense transcripts are of particular interest given that *Cryptosporidium* lacks typical dsRNA degradation machinery such as Dicer and Argonaute. To further investigate the role of dsRNAs in *Cryptosporidium* gene regulation, dsRNA-seq datasets were generated by extracting total RNA from *Cryptosporidium* sporozoites and enriching for dsRNA using immunoprecipitation with the J2 antibody. Sequencing data were analyzed to a) confirm that *Cryptosporidium* sporozoites do contain dsRNA, and b) determine which genes are covered by the reads. Further research will be directed towards understanding whether dsRNAs in *Cryptosporidium* have a biological role.

P12. Development of a cell synchronization protocol for the brain-eating amoeba, *Naegleria fowleri*

Anissa J. Waller Del Valle[1,2], Dennis E. Kyle[1,2,3]
[1]Center for Tropical and Global Emerging Diseases, Athens GA; [2]Department of Cellular Biology, University of Georgia, Athens, GA; [3]Department of Infectious Diseases, University of Georgia, Athens, GA

Naegleria fowleri, commonly referred to as the brain-eating amoeba, is a free-living amoeba and early-diverged excavate that serves as the causative agent of primary amebic meningoencephalitis (PAM). PAM is an incredibly rare, progressively fatal disease with a fatality rate upwards of 97%. Despite its medical significance, many fundamental aspects of its cellular biology remain poorly understood – including its potential for sexual reproduction. It is known that *Naegleria* trophozoites replicate primarily through a primitive form of intranuclear cellular division known as promitosis. However, recent genomic evidence found that several species of *Naegleria,* including *N. fowleri* and *N. gruberi*, harbor a meiotic toolkit. The meiotic toolkit comprises genes involved in meiotic and non-meiotic processes, as well as genes that are involved exclusively in meiosis. This is furthered by the presence of meiosis-specific structures, such as the karyosome, as well as structures that are dependent on sexual reproduction for their function. We aim to develop a cell synchronization protocol to facilitate the discovery of a meiotic life stage within *N. fowleri*, as cell synchronization would allow us to address various aspects of *Naegleria*'s reproduction. To attempt this, cells were treated with aphidicolin, a DNA polymerase α inhibitor, to synchronize them to the G1/S phase border. Cells were then treated with RO-3306, a CDK-1 inhibitor, to further synchronize them to the G2/M phase border. Synchronization efficiency was determined by flow cytometry. Altogether, this work will aid in ongoing studies to determine if *N. fowleri* possesses a meiotic life stage.

P13. A nuclear protein with YqgF1 and SH2 domains regulates S-phase in *Trypanosoma brucei*

Benjamin Hoffman[2], Catherine Sullenberger[2] and Kojo Mensa-Wilmot[1,2,3]
[1]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA 30144,
[2]Department of Cellular Biology, University of Georgia, Athens, GA 30602, [3]Center for Tropical and Emerging Global Diseases, UGA, Athens, GA 30602

Hallmarks of S-phase in the unicellular eukaryote *Trypanosoma brucei* include basal body (centriole) duplication and nuclear DNA synthesis. Casein kinase CK1.2 regulates S-phase because its knockdown leads to (i) DNA synthesis in post-mitotic trypanosomes that are not scheduled to be replicating their genome, (ii) runaway duplication of basal bodies and (iii) dephosphorylation of histone H2A. Effects of CK1.2 knockdown on basal body duplication and DNA synthesis are phenocopied by addition of SB-431542, an inhibitor of CK1.2, to *T. brucei*. Signaling pathways for CK1.2 are not well-defined, possibly because of extensive divergence of trypanosome protein sequences from those of commonly studied eukaryotes. To identify proteins that could mediate signaling by CK1.2 we screened polypeptides that were (a) dephosphorylated after knockdown of the kinase, (b) localized to the nucleus, and (c) essential for proliferation of the trypanosome. Included in the three proteins identified with this workflow was hypothetical polypeptide Tb927.02.5810 that contains SH2 and YqgF domains. Consistent with a hypothesis that Tb927.02.5810 mediates signaling by CK1.2, knockdown of its gene inhibits DNA synthesis, increases basal body copy number, and increased phosphorylation of H2A. These results document importance of Tb927.02.5810 for S-phase in *T. brucei* and validate a strategy of using a protein kinase to guide functional annotation of a hypothetical protein.

P14. Identifying the mechanism of action of a novel antimalarial with collateral drug sensitivity associated with *Pf*Kelch13 C580Y mutation

Reagan S. Haney[1,2], Leticia Do Amaral[1,2], Jopaul Mathew[3,4], Emilio F. Merino[1,2], Maxim Totrov[7], Paul R. Carlier[5,6], Maria B. Cassera[1,2]
[1]Department of Biochemistry and Molecular Biology, [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, USA;[3]Department of Chemistry [4]Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, Virginia, USA; [5]Department of Pharmaceutical Sciences [6]Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois, USA; [7]Molsoft LLC, San Diego, California, USA

Malaria is a devastating disease that caused approximately 597,000 deaths in 2023 worldwide. Cases of malaria have increased from previous years in part by the quick development of resistance to current antimalarials. Resistance to antimalarial drugs, such as artemisinin and its derivatives, creates 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 (PRC1584) discovered by our research team. During our investigation to identify the molecular target(s) and the mechanism of action (MOA) of PRC1584, we discovered that it has collateral drug sensitivity with one of the known mechanisms of resistance to dihydroartemisinin (DHA). DHA resistance can be conferred by a single nucleotide polymorphism (SNP) in the *PfKelch13* gene known as Kelch13 (K13) and a few SNPs have been reported, with C580Y and R539T being the most relevant mutations. Preliminary data indicate that parasites carrying a K13 C580Y mutation are more susceptible to PRC1584 treatment and disrupts hemoglobin uptake and metabolism. Therefore, we hypothesize that K13 and/or its interactors may be molecular targets of PRC1584. We selected *Plasmodium falciparum* DHA-sensitive and DHA-resistant strains with K13 C580Y mutations and assessed PRC1584 EC_{50} values, hemoglobin digestion, and evaluated cytostome formation after PRC1584 treatment. Altogether, these experiments and ongoing chemoproteomics studies will reveal if K13 or its interactors are the molecular target(s) or are involved in the MOA of PRC1584. Identifying the MOA of PRC1584 will guide its pre-clinical development to prevent late-stage failure.

P15. Chemical and genetic validation of an essential calcium entry channel of *Trypanosoma brucei* as a therapeutic target

Guozhong Huang[1], Harmanpreet Singh[2], Priti Singh[2], Rohit Vashnaya[2], Donald Hamelberg[2], Binghe Wang[2] and Roberto Docampo[1]

[1]Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602. [2]Department of Chemistry, Georgia State University, Atlanta, GA 30303

The *Trypanosoma brucei* group of parasites causes Nagana in cattle and Human African trypanosomiasis (HAT), or sleeping sickness, in humans. Current drugs against these parasites have severe toxicity, vaccines are not available, and development of drug resistance makes finding new chemotherapeutic targets imperative. Ion channels, which are involved in several biological processes, are targets of many therapeutically useful agents and they remain significantly underexplored as therapeutic targets in parasites. Here we report the presence of a voltage gated Ca^{2+} channel (VGCC, $TbCa_v$), which is localized in the flagellar plasma membrane of *T. brucei* and is essential for proliferation of both bloodstream (BSF) and procyclic forms (PCF) of the parasite. $TbCa_V$ is a single subunit channel capable of transporting Ca^{2+} when expressed in mutant yeast lacking plasma membrane Ca^{2+} channels or in HEK293T cells. Through virtual screening of a commercial chemical library using dynamic ensembles of various conformations of $TbCa_v$ and associated docking analyses, several inhibitors of $TbCa_v$ were discovered. As pharmacological validation of the essential roles of $TbCa_v$, these inhibitors were shown to inhibit *T. brucei* growth with the most potent agent, N-(7-nitro-2,1,3-benzoxadiazol-4-yl) acetamide (NBD-A), exhibiting an EC_{50} of 25 ± 3 nM and no cytotoxicity in Vero cells possessing related channels. Thus, such studies constitute a pharmacological validation of $TbCa_v$ as a viable therapeutic target of *T. brucei*.

P16. Visualization and quantification of ART-induced dormant *P. falciparum* using cytoplasmic markers

Victoria Mendiola[1], Dr. Dennis Kyle[1]

[1] Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA

Artemisinin-based combination therapies (ACTs) are the frontline treatment for *Plasmodium falciparum* malaria. However, the emergence of artemisinin resistance, characterized by delayed parasite clearance, threatens malaria control efforts. Artemisinin (ART)-induced dormancy, where early ring-stage parasites survive by entering a reproductively inactive, condensed nuclear, and reduced metabolic state, contributes to ACT treatment failure. Dormant parasites morphologically resemble dead parasites, complicating their identification using common diagnostic methods, such as Giemsa-stained blood smears. This study aims to identify novel methods for visualizing and enumerating ART-treated *P. falciparum* by exploiting the distinct biology of dormant parasites. ART-sensitive and ART-resistant *P. falciparum* strains were stained daily for five days post-drug exposure using Hoechst 33342 (nuclear stain) and CellTracker Green CMFDA (cytoplasmic stain). Parasite morphology and staining patterns were assessed by 100x fluorescence microscopy and flow cytometry. These results show both dead and dormant parasites retained nuclear staining, limiting differentiation using Hoechst staining alone. However, dormant parasites exhibited reduced but detectable cytoplasmic staining, thus providing a more reliable method for identifying and quantifying dormant parasite populations using a dual staining method. This work establishes a novel approach for visualizing and quantifying ART-induced dormant parasite recovery, providing a foundation for the development of more effective assays to elucidate the biology of ART-induced dormant parasite recovery.

P17. Potency, plasticity, and diversity of stem cells in the rat tapeworm, *Hymenolepis diminuta*

Corey W. Rennolds[1,2], Tania Rozario[1,2]
[1]Department of Genetics, UGA, Athens, GA; [2]CTEGD, UGA, Athens, GA

Tapeworms are successful parasites due in part to their rapid growth, shedding of reproductive segments called proglottids, and subsequent regeneration of proglottids. The cellular and molecular basis of such continuous, large scale tissue turnover remains poorly understood. The rat tapeworm, *Hymenolepis diminuta*, contains a sole population of proliferative cells with body-wide distribution that are required for growth and regeneration, like planarian neoblasts, indicative of stem cells (SCs). Unlike planarians, *H. diminuta* regeneration is not body-wide, consisting only of proglottid regeneration from the neck. Understanding this regenerative ability requires isolating and characterizing the SCs, including determining their potency, diversity, and developmental relationships. We are conducting parallel approaches to isolate SCs. First, we are using basic stains and fluorescence activated cell sorting (FACS) to enrich for SCs. We have found three populations of cells differing in nuclear DNA content, possibly corresponding roughly to cell cycle stages, and we plan to use single-cell RNA-seq (scRNA-seq) of the putative 4C cells to distinguish SC subpopulations, including any pluripotent cells and lineage-restricted progenitors that may exist. Second, we are using existing scRNA-seq datasets to discover SC markers, including cell surface receptors that may serve as antibody targets for FACS. We anticipate that these approaches will yield novel insights regarding the composition of tapeworm SCs and facilitate further work to understand SC function, potency, and plasticity in the context of development.

P18. Unique interactions between the succinate dehydrogenase and the ubiquinone biosynthesis complex in *Toxoplasma gondii*

Baihetiya Baierna[1,2], Silvia NJ Moreno[1,2]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, USA,
[2]Department of Cellular Biology, University of Georgia, Athens, Georgia, USA

Toxoplasma gondii is an apicomplexan parasite that infects approximately one third of the world population and causes serious diseases in immunosuppressed individuals. These parasites possess a single mitochondrion and one of the major anti-toxoplasma drugs, atovaquone, is a competitive inhibitor of ubiquinol and blocks the mitochondrial electron transport chain (ETC) at the cytochrome bc1 site. We characterized the *T. gondii* synthesis of ubiquinone (UQ) and discovered that several of the enzymes catalyzing the UQ ring decorations are arranged in a large protein complex. We identified five proteins (TgCoq3, TgCoq4, TgCoq4, TgCoq8 and TgCoqFAD) that form part of the UQ complex in *T. gondii*. These enzymes were very divergent from the mammalian counterparts and most interestingly, TgCoqFAD was of plant origin and most likely catalyzing more than one activity. Interestingly, the size of the complex was much larger than the ones present in other organisms. Mass spectrometry analysis of samples obtained after proximity labeling combined with subcellular fractionation of a mutant expressing TgCoq4-TurboID revealed proteins from the succinate dehydrogenase complex (CII) significantly enriched. Following on this finding we performed membrane-based yeast two hybrid in addition to reciprocal co-immunoprecipitation with endogenous proteins and demonstrated that TgCoq4 and TgCoq5 interact with CII subunits and that the interaction is specific to CII apicomplexan subunits. Using Blue Native PAGE and 2-dimensional PAGE assays, we discovered that the interaction between the UQ complex and the CII is unique to *T. gondii* and could not be demonstrated in mammalian cells. We also showed that this interaction was important for the CII activity. Our work presents a novel super complex interaction that has not been demonstrated in any organisms to date.

P19. The enigmatic mitochondrial genome of *Sarcocystis neurona*

Rafeed R. Turjya[1], George L. Kitundu[1,2], and Jessica C. Kissinger[1,3,4]
[1]Institute of Bioinformatics, University of Georgia, [2]Department of Biochemistry and Biotechnology, Pwani University, Kenya, [3]Center for Tropical and Emerging Global Diseases & [4]Department of Genetics, University of Georgia, Athens, GA, USA

Among apicomplexans, *Toxoplasma gondii* and other closely related genera exhibit fragmented mitochondrial genomes (mtDNA). The fragments, designated as sequence blocks (SBs), are redundantly present in specific orientations in the mtDNA, identifiable with long read sequencing. But mtDNA fragmentation is absent in most other apicomplexans e.g., *Eimeria tenella* and *Plasmodium spp.* With unknown mitochondrial genome sequence and topology, *Sarcocystis neurona,* the causative agent of equine protozoal myeloencephalitis (EPM), falls between *T. gondii* and *E. tenella* on the phylogenetic tree. To resolve the genome, Oxford Nanopore (ONT) reads were generated from *S. neurona* parasites grown in *Bos taurus* cell culture. Reads mapping to host genome as well as *S. neurona* nuclear and apicoplast genomes were sequentially filtered out. The remaining reads were mapped to coding sequences of known *S. neurona* mitochondrial genes. Mapped reads were used to identify 18 unique SBs with a total length of 5960 bp, which combine in 24 defined block orders. 3661 ONT reads mapping to the SBs were identified as mitochondrial, with most of them shorter than 1000 bp. Microhomologies are abundant within the *S. neurona* SBs, but none longer than 15 bp. There is significant divergence in *S. neurona* SBs from *T. gondii*, but a pair of SB boundaries are conserved - indicative of the initial mtDNA fragmentation prior to the divergence of *S. neurona* and *T. gondii.* The three mitochondrial protein encoding genes show different fragmentation patterns and variable conservation across species. Fragmentation in *S. neurona* mtDNA implies unexplored molecular processes with potential therapeutic value.

P20. Generating an episomally maintained transgene vector in *Naegleria fowleri*

Colm Roster, Jillian McKeon, and Jim Morris
Eukaryotic Pathogen Innovation Center, Department of Genetics and Biochemistry, Clemson University

Naegleria fowleri is a pathogenic free-living amoeba and the causative agent of primary amebic meningoencephalitis (PAM), an infection that occurs when amoeba infiltrates the brain following exposure of contaminated water. Coupling the limited diagnostic methods with the paucity of effective therapeutic intervention, PAM has a >97% mortality rate. Manipulating gene expression has become a principal technique for generating effective and specific novel therapies. However, the tools required to complete these tasks in *N. fowleri* have not yet been developed. Here, we describe work to generate an artificial *N. fowleri* chromosome using replication and maintenance sequences from a *Naegleria* episome called the closed extrachromosomal ribosomal DNA element (CERE). Little is known about the CERE sequence of the genome reference *N. fowleri* Ty strain, and important replicative or regulatory sequences remain unresolved. Following the development of methods for the isolation of CERE from whole-cell lysate, we have generated partial sequences of the episome using PCR with primers designed based on CERE sequences from other *N. fowleri* strains. These fragments are now being used to generate fusion plasmids within a standard bacterial vector in transfection experiments to score whether a fragment is sufficient to promote the propagation of the recombinant plasmid. Because CERE DNA elements involved in replication likely engage CERE-specific proteins that could be interesting drug targets, we have also worked to isolate CERE-associated proteins using pulldown approaches, which will be described. Further characterization of CERE DNA and protein factors that govern its replication will direct the generation of heritable transgene constructs while also shedding new insight into the biology of this unique DNA element.

P21. Utilizing the ATP FRET sensor ATeam3.10 to quantify mitochondrial ATP concentration changes in *Toxoplasma gondii*

Aidan May[1] and Diego Huet[1,2]
[1]CTEGD, UGA, Athens, GA; [2]Department of Pharmaceutical and Biomedical Sciences, UGA, Athens, GA

Förster resonance energy transfer (FRET) sensors are non-radiative biosensors capable of detecting changes in biomolecule concentrations within live samples via the utilization of twin fluorophores. These fluorophores act as a donor/acceptor system, with the donor's fluorescent emission being constitutively expressed under standard conditions, whereas the acceptor's fluorescence is triggered by a conformational change in the protein. This change is triggered by a specific activator to which the protein has high affinity for, permitting the detection and quantification of the activator's concentration via the acceptor's fluorescent intensity. This model has been utilized to live visualize mitochondrial ATP concentrations within live mammalian cells under several conditions, though similar studies have not been successfully conducted in *Toxoplasma gondii.* Despite the FRET system's versatility and ability to monitor concentration changes in live cells, there is currently not an ATP FRET sensor adapted to *T. gondii*, with current concentration quantification methods being terminal and producing a single timepoint. Previous work by the Huet Lab has seen the ATP FRET sensor ATeam1.03 adapted to *T. gondii*, however it lacked the dynamic range necessary to track mitochondrial ATP concentration changes. ATeam3.10 is a variant of ATeam1.03 possessing a greater affinity for ATP, making it an ideal candidate to track mitochondrial ATP concentration in *T. gondii.* Here, we utilize ATeam1.03-YEMK, another variant of ATeam1.03, as a vector backbone to adapt ATeam3.10 to *T. gondii*. This will permit future work quantifying mitochondrial ATP concentration changes following modification of ATP synthase subunits and ATP production pathways, allowing us to determine significance to ATP production.

P22. Exploring dedaquiline as an apicomplexan ATP synthase inhibitor

Samuel Nyarko[1,2], Anthony Roberto[1] Douglas Graeme Paton[3] and Diego Huet[1,4]
[1]CTEGD, UGA [2]Cellular Biology, UGA, [3]Infectious Diseases UGA, [4]Pharmaceutical & Biomedical Sciences
Athens, GA

Toxoplasma gondii is one of the most successful apicomplexan parasites that infects an estimated 30% of the global population. The current treatment options for toxoplasmosis are ineffective, necessitating the development of new therapies with independent modes of action to overcome the therapeutic obstacles posed by drug resistance and the resilience of *Toxoplasma* chronic stages. Our approach capitalizes on the unique apicomplexan biology, revealing a compelling avenue for repurposing existing drugs to accelerate the development of effective treatments. The *T. gondii* mitochondrial ATP synthase is highly divergent, possessing 17 unique subunits and apicomplexan-specific extensions. We hypothesize that exploiting these features could aid in developing the novel therapies. In this work, we aim to investigate the mechanism by which bedaquiline (BDQ), a mycobacterial ATP synthase inhibitor, affects apicomplexans. Here, we show that BDQ inhibits *T. gondii* growth and ATP production via the ATP synthase. To identify the molecular target of this drug, we generated BDQ-resistant *T. gondii* lines through chemical mutagenesis. Resistant clones were confirmed to have a higher EC50 values compared to wildtype parasites. Whole-genome sequencing reveals candidate gene mutations in proteins involved in gene expression regulation, mitochondrial function and mitochondrial RNA metabolism. In our ongoing research, we seek to elucidate how these candidate mutations confer BDQ resistance, specifically addressing whether BDQ's inhibition of the *T. gondii* ATP synthase is a direct or indirect effect. Our results will provide a foundation for investigating how inhibitors target the unique apicomplexan mitochondrial ATP synthase.

P23. Lead optimization and target identification of a new series of antimalarial compounds targeting *Plasmodium vivax* hypnozoites: opportunities and challenges

Anthony Ruberto[1], Yingzhao Zhao[2], Lili Huang[2], Sagan De Castro[1], William Long[1], Camille Roesch[3], Ami H. Asakawa[2], Chungsik Kim[2], Paul Willis[4], Brice Campo[4], Zaira Rizopoulos[4], Amélie Vantaux[3], Steven Maher[1], Benoit Witkowski[3], Roman Manetsch[2], Dennis Kyle[1]

[1] Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, [2] Center for Drug Discovery, Northeastern University, Boston, MA, [3] Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [4] Medicines for Malaria Venture, Geneva, Switzerland

Malaria caused by *Plasmodium vivax* is a neglected tropical disease that infects up to 15 million people each year, with ~3 billion people at risk. *P. vivax* hypnozoites are the dormant parasite reservoir in the liver that causes secondary infections called relapses. Relapsing infections are responsible for the majority of vivax malaria cases worldwide. Currently only two drugs—primaquine and tafenoquine— are approved to prevent relapse, but both drugs are 8-aminoquinolines that can't be administered to patients with specific genetic backgrounds. New, safe and effective therapies are needed. However very little is known about the biochemical pathways that are active in hypnozoites; and furthermore, there are no validated drug targets. Using a medium throughput assay for *P. vivax* liver stages in vitro, we identified a new non-8-aminoquinoline compound (MMV987) with hypnozonticidal activity. We have performed optimization of the MMV987 hit series for improved potency, stability, and bioavailability. Current efforts involve identifying the mechanism of action and target(s) of our hit compound using various omics-based approaches, including chemo-genomics and chemo-proteomics. The output of these data point toward a connection between MMV987 and pathways associated with protein degradation and suggest that the disruption of protein homeostasis negatively impacts parasite survival. We highlight the opportunities and challenges associated with compounds development targeting *Plasmodium vivax* liver forms, including clinically silent hypnozoites.

P24. Discovery and characterization of overlapping chromosome 10 copy number variance in multiple in vitro selected artemisinin resistant *Plasmodium falciparum*

Zhe Cheng[1,2], Anthony A. Ruberto[1], Matthew Tucker[5], James Oristian[1,3], Rodrigo Baptista[5], Mingyu Wang[4], Carrie Brooks[1], Vasant Muralidharan[1,2], Dennis E. Kyle[1,2,3]

[1]Center for Tropical and Emerging Global Disease, University of Georgia, Athens, GA, [2]Department of Cellular Biology, University of Georgia, Athens, GA, [3]Department of Infectious Disease, University of Georgia, Athens, GA, [4]Institute of Bioinformatics, University of Georgia, Athens, GA, [5]USDA Agricultural Research Service, Beltsville, MD, [6]Houston Methodist Research Institute, Houston, TX

Artemisinin resistance is an important global health issue, reflected by frequent reports of clinical treatment failure and delayed parasite clearance. It has been shown that artemisinin induced dormancy is a major mechanism for parasites to survive artemisinin exposure. To further understand the underlying molecular mechanisms, we characterized two previously selected artemisinin resistant *P. falciparum* D6R and W2R along with their sensitive parentals through genomic and transcriptomic approaches. Whole genome sequencing revealed an overlapping amplification of 10 genes on chromosome 10 in both artemisinin resistant clones, in the absence of known resistance markers including K13 mutations. Single cell RNA sequencing showed tight transcriptional regulation of expression profile of genes inside chromosome 10 CNV in accordance with copy number amplification in D6R. Overexpression of 5 candidate genes from the chromosome 10 CNV resulted in variable effects on parasite recovery from artemisinin exposure. Collectively, these findings suggest that chromosome 10 CNV may serve as a novel marker of early adaptation to increased artemisinin resistance.

P25. Nested genes of apicomplexan parasites and a potential 'Tag-in-Place' Strategy

Chasen NM[1], Lechtreck KF[1]
[1]Department of Cellular Biology, University of Georgia, Athens, GA 30602

The complex arrangement of genes in eukaryotic organisms has been well established over several decades. One particularly interesting case is when gene-coding regions overlap and allow for a single genomic DNA region to encode for two or more separate gene products. In this work, we mined the annotated genomes of apicomplexan parasites to identify nested genes, followed by an analysis to quantify their number, along with characteristics such as size and directionality. Cartoon representations are included, showing notable examples of these overlapping genes from each organism. Additionally, we put forward our work with the unicellular algae *Chlamydomonas reinhardtii,* as proof of concept for a nested gene based endogenous 'Tag-in-Place' system that allows for drug selection, while still preserving native gene elements such as promoters, signal peptides, and UTRs.

P26. Increased Duffy binding protein 1 expression correlates with *Plasmodium cynomolgi* growth in continuous culture

Wayne T. Cheng[1,2,] Magdalena Argomaniz[1,2], Caitlin C. Cooper[2], Amadis Vivas[1,2],Saniya Sabnis[1,2], Sarah Gayle Roberson[1,2], Celia L Saney[1,2], Mary R. Galinski[1,2], Steven P. Maher[2], Dennis E. Kyle[2,3], Chester J. Joyner[1,2,3]
[1]Center for Vaccines and Immunology, College of Veterinary Medicine, UGA; Athens, GA, 30602, USA. [2]Center for Tropical & Emerging Global Disease, UGA; Athens, GA, 30602, USA. [3]Department of Infectious Diseases, College of Veterinary Medicine, UGA, Athens, GA, 30602

A continuous culture system would revolutionize *Plasmodium vivax (Pv)* research but remains elusive. *Plasmodium cynomolgi (Pcy)* is a closely-related nonhuman primate malaria parasite that shares many biological traits with *Pv* except that *Pcy* preferentially, but not exclusively, invades and develops within reticulocytes. This difference has supported the adaptation of *Pcy* lines that grow in culture, but the mechanisms that enable continuous culture are undefined. Here, we generated a new line of the *Pcy* Berok strain, termed DC line, to grow continuously in culture and performed whole genome sequencing of parasites collected during adaptation to identify the genetic changes that promote growth in culture. Minimal single nucleotide variants emerged during adaptation. Structural variations comprised of insertions and deletions (INDELs) were more common and suggested that a subpopulation of parasites was selected for during adaptation versus de novo mutations that led to improved growth. INDELS were present in many genes associated with the parasite's metabolism, consistent with the nutrient-limited environment of culture. Interestingly, the DC line also had additional copies of the Duffy binding protein 1 gene that was associated with increased gene expression. Duffy antigen receptor for chemokines (DARC) is the ligand for DBP1, and the loss of this receptor has been shown to restrict *P. yoelii* to invading reticulocytes. Thus, we hypothesized that overexpression of DBP1 by the DC line may alter the invasion preference from reticulocytes to normocytes, enabling the parasite to grow effectively in culture. Indeed, invasion assays showed that the WT line preferentially invaded and developed within reticulocytes whereas there was no preference for the DC line. In summary, these data indicate that metabolic changes and alterations in invasion ligand expression through copy number variation support continuous growth of *P. cynomolgi* in culture. This information may help adapt additional *Pcy* strains in vitro and inform efforts for culturing *Pv*.

P27. A *Plasmodium vivax* strain that expresses fluorescent proteins throughout the life cycle

Magdalena Argomaniz[1,2], Wayne T. Cheng[1,2], Amadis Vivas[1,2], Grace Hawkins[1,2], Henry R. Davie[1,2], Sarah Gayle Roberson[1,2], Vasant Muraldiharan[2], Diego Huet[2] , Steven P. Maher[2], Chester J. Joyner[1,2]

[1]Center for Vaccines and Immunology, College of Veterinary Medicine, University of Georgia; Athens, GA, 30602, USA. [2]Center for Tropical & Emerging Global Disease, University of Georgia; Athens, GA, 30602, USA

Plasmodium vivax (*Pv*) persists due to its ability to form dormant liver-stages, known as hypnozoites (HZs). Understanding the molecular makeup of HZs is key to developing new treatments to eliminate HZs, but these experiments have been hindered by the inability to isolate *Pv* HZs for molecular characterization. A transgenic *Pv* that expresses fluorescent proteins throughout the life-cycle would overcome this limitation and make molecular characterization possible. To address this need, *Pv* Chesson parasites were harvested from *Saimiri boliviensis* monkeys and transfected with a plasmid containing *gfp, mCherry*, and *nanoluc* reporter genes under two different promoters. GFP was placed under the constitutively expressed *hsp70* promoter, whereas mCherry and Nanoluc were placed under the *lisp2* promoter to enable the exclusion of activating forms from dormant HZs in future isolations. Pyrimethamine resistant asexual stage parasites were recovered about 31 days after transfection and inoculation into a naïve animal. Eighty-nine percent of the resistant parasites expressed GFP. Infected blood was then collected and fed to *Anopheles stephensi* mosquitoes, and GFP+ oocysts and sporozoites were detected. Primary human hepatocyte cultures were inoculated with sporozoites, and both small and large forms expressing GFP were detected by live imaging. Large forms also expressed mCherry as expected. There were no effects on the parasite's development in the liver-stages. This study establishes a fluorescent, transgenic *P. vivax* strain that can be used to isolate hypnozoites for molecular characterization and methods for genetically manipulating *P. vivax* to test RNA binding proteins that may be involved in dormancy.

P28. Resolving the function of the SET domain protein lysine methyltransferase in *Trypanosoma brucei*

Hannah Abbey[1], Emily W. Knight[1], Bryson Woodard[1], Meredith Morris[1]

[1]Eukaryotic Pathogen Innovation Center, Department of Genetics and Biochemistry. Clemson University

Kinetoplastid parasites cause major public health and economic burdens in sub-Saharan Africa, the Middle East, and the Americas. Limited treatment options and rising drug resistance highlight the need for novel parasite-specific therapeutic targets. SET domain protein lysine methyltransferases (PKMTs) transfer methyl groups from donors like S-adenosyl-L-methionine to lysine residues. The *T. brucei* genome encodes seven putative SET domain proteins that lack homologs in other organisms. One of these, Tb927.9.11350 (TbSETD) is essential, localizes to mitochondria, and RNA interference results in mitochondrial swelling and dysfunction. TbSETD binding partners are enriched in mitoribosome and mitoribosome accessory proteins. *In vitro*, TbSETD methylates proteins from parasite lysate at 70 kDa and 35kDa. In gel slices, mass spectrometry reveals 50 proteins. Fifteen of them are mitochondrial and include two mitochondrial accessory proteins. Four additional SET proteins (Tb927.1.2730, Tb927.3.810, Tb927.4.1260, Tb927.7.2040) are mitochondrial-localized in TrypTagDB, and enriched in mitoribosome proteomes, suggesting they may share a common function in parasite biology. We hypothesize that kinetoplast-specific SET domain proteins regulate mitoribosome assembly and mitochondrial gene expression, impacting parasite metabolism. Current work is focused on identifying the specific substrates of the SET domain proteins and resolving the mechanisms by which they regulate mitochondrial gene expression. Given their essentiality and druggability, these proteins represent promising targets for anti-parasitic drug development. Tb927.1.2730 is another essential, kinteoplastid-specific SET domain PKMT. Preliminary imaging shows that Tb927.1.2730-deficient cells lack kDNA.

P29. Redox regulation of calcium homeostasis in *Toxoplasma gondii* for optimal lytic cycle progression

Katherine E. Moen, Silvia NJ Moreno
CTEGD, UGA, Athens, GA Dept. Cellular Biology, UGA, Athens, GA

The Apicomplexan parasite, *Toxoplasma gondii*, infects approximately one third of the world population, posing a significant risk to immunosuppressed individuals and unborn fetuses. Its fast-replicating tachyzoite form engages in a lytic cycle, causing host tissue damage and contributing to pathogenesis. Calcium plays a crucial role in driving the lytic cycle, and the precise regulation of calcium within the parasite is essential for the successful progression of the cycle. Cytosolic calcium is tightly controlled, either sequestered in intracellular stores or pumped out of the cell. The endoplasmic reticulum (ER) serves as the largest intracellular store of calcium in most eukaryotic cells, and calcium is important for many ER functions. Additionally, the ER maintains an oxidative environment that promotes disulfide bond formation, a critical process for proper protein folding. Protein disulfide isomerases (PDIs) are ER redox enzymes that facilitate the formation, breakage, and rearrangement of disulfide bonds between cysteine residues, thereby stabilizing protein structures or modulating protein function. Endoplasmic reticulum oxidoreductin 1 (ERO1) is an ER redox enzyme that is important from removing disulfide bonds from PDIs in order to maintain their catalytic function. In this study, we investigate the ER redox enzymes TgPDIA3, an essential *T. gondii* PDI, and TgERO1, *T. gondii*'s resident ER oxidoreductase. We explore their roles in the redox regulation of calcium sequestration by the Endoplasmic Reticulum Calcium ATPase (SERCA) in the *T. gondii* ER, and assess how these functions contribute to the parasite's lytic cycle.

P30. Comprehensive QTL mapping in a Kelch13 wildtype *Plasmodium falciparum* genetic cross

Mackenzie A.C. Sievert[1] and Katelyn Vendrely Brenneman[2], Lisa A. Checkley[3], Tess Marvin[4], Megan Schmidt[4], Douglas A. Shoue[3], Gabriel J. Foster[3], Sudhir Kumar[5], Xue Li[6], Marina McDew-White[6], François H. Nosten[7,8], Ian H. Cheeseman[6], Ashley M. Vaughan[9], Timothy J.C. Anderson[6], Jeanne Romero-Severson[3], Michael T. Ferdig[3]
[1]CTEGD, UGA, Athens, GA [2]Harvard University, Cambridge, MA [3]University of Notre Dame, South Bend, IN [4]Princeton University, Princeton, NJ [5]Iowa State University, Ames, IA [6]Texas Biomedical Research Institute, San Antonio, TX [7]Shoklo Malaria Research Unit, Mahidol University, Mae Sot, Thailand [8]Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK [9]Seattle Children's Research Institute, Seattle, WA

Plasmodium falciparum genetic crosses suitable for Quantitative trait locus (QTL) mapping have led to key discoveries of genes involved in many specific phenotypes. Utilizing a human liver-chimeric mouse infused with human red blood cells (the FRG huHep/huRBC mouse), we generated progeny from a cross between an artemisinin resistant isolate from Southeast Asia with no resistance-associated kelch13 mutation, NHP4026, and the common lab strain, NF54. We phenotyped this progeny set for multiple phenotypes including i) extended recovery ring stage survival assay (eRRSA), ii) competitive growth as a surrogate for fitness in RBCs, iii) dose responses to four antimalarial compounds and iv) genome-wide transcription profiles at three timepoints. Candidate genes identified through our integrated data acquisition and analysis will help to investigate the underlying genetics of multidrug resistance, compensatory mutations that allow drug resistance to spread, and how these genetic changes affect a broad range of biological processes. These data demonstrate that new crosses using recent patient-derived isolates, and a comprehensive phenotyping approach can capture the genetic basis of emerging drug resistance in real time.

P31. Stage-specific and temperature-responsive control of protein stability in *Trypanosoma cruzi* using a DHFR destabilizing domain system

Ganesh Babu Malli Mohan[1], Justin Wiedeman[1], Ruby Harrision[1], and Ronald Drew Etheridge[1,2]
[1]CTEGD, UGA, Athens, GA, [2]Dept. of Cellular Biology, UGA, Athens, GA

Destabilizing domains (DDs) enable ligand-inducible, temporal control of protein abundance, offering a valuable tool for functional studies. The protozoan parasite *Trypanosoma cruzi,* the causative agent of Chagas disease, poses a significant public health challenge across the Americas. Despite its clinical importance and complex life cycle involving distinct temperature-dependent stages, genetic tools for conditional protein regulation in *T. cruzi* remain limited. To address this gap, we adapted a mutant *Escherichia coli* Dihydrofolate Reductase (DHFR)-based DD system to achieve regulated protein degradation in response to physiologically relevant temperature shifts. We fused the DHFR-DD to the essential mitotic regulator Aurora Kinase and introduced the construct into *T. cruzi* cells. Our results demonstrate effective, ligand-dependent degradation of the fusion protein, enabling conditional knockdown in both the epimastigote and intracellular amastigote stages. These findings highlight the potential of DHFR-DD systems for stage-specific functional studies in *T. cruzi* and pave the way for broader applications in protozoan parasites undergoing temperature-dependent transitions.

P32. Two historical 4(1H)-quinolone scaffolds have potent efficacy against acute and chronic stages of *Toxoplasma gondii*

Melissa A. Sleda[1], Khaly Diagne[2], Baihetiya Baierna[1,3], Victoria Mills Clifton[3], Roman Manetsch[2], Silvia NJ Moreno[1,2]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, USA, [2]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA, [3]Department of Cellular Biology, University of Georgia, Athens, GA, USA

Toxoplasma gondii is a protozoan parasite that can infect many warm-blooded animals, including humans and can cause devastating disease in immunocompromised individuals and the unborn fetus. The current treatments against toxoplasmosis are only effective against the acute stages with little effect against bradyzoites found in tissue cysts. The mitochondrion of *T. gondii* is a validated target and one of the major anti-toxoplasma drugs, atovaquone, inhibits the mitochondrial electron transport chain (ETC) through inhibition of the coenzyme Q:cytochrome c oxidoreductase. Herein, we test two 4(1H)-quinolone scaffolds that have previously been shown to be inhibitors of the mitochondrial bc_1 complex in *Plasmodium falciparum*. We show that the two compounds, ICI-56780 and WR-243246 inhibit the growth of tachyzoites with low nanomolar EC_{50}s. We also show that both compounds inhibit cytochrome c reduction and mitochondrial membrane potential in *T. gondii*. Additionally, the ICI-56780 protects mice against a lethal dose of RH tachyzoites. Most importantly, we show that ICI-56780 is effective against bradyzoites *in vitro,* reducing both the cyst size and bradyzoite viability. ICI-56780 also inhibits the viability of *in vivo* derived bradyzoites with low nanomolar EC_{50}, and significantly decreases tissue cyst burden in mice chronically infected with *T. gondii*. This preliminary testing of the 4(1H)-quinolone scaffolds show promising results for treating chronic toxoplasmosis. Although these scaffolds have some pharmacokinetic limitations, the findings provide valuable insights for guiding the future synthesis of analogs to improve pharmacokinetics while maintaining potent efficacy against chronic Toxoplasmosis.

P33. Additional blood meals after infection increase fitness of malaria parasites and their mosquito host

Phipps BL[1], Shiau JC[2], Flentje JP[3], Sharma R[2], Pathak AK[2], Brown MR[3], Strand MR[3].
[1]Department of Genetics, [2]Department of Infectious Diseases, [3]Department of Entomology University of Georgia, Athens, GA

Female mosquitoes blood-feed to acquire nutrients for egg production, often doing so multiple times to produce multiple clutches of eggs. This provides an opportunity for certain blood-borne pathogens to spread between vertebrate hosts. *Anopheles* mosquitoes transmit malaria parasites, which belong to the genus *Plasmodium* and cause nearly half a million human deaths annually. Despite their virulence to vertebrates, several studies have demonstrated that these parasites impose no fitness cost on their vector while exploiting blood meal-derived nutrients for their own development. Furthermore, an additional blood meal after infection can accelerate *P. falciparum* development in *A. gambiae*, suggesting a second influx of nutrients during *Plasmodium* development can benefit transmission potential. However, whether the number and timing of blood meals shapes the parasite's development and interaction with the mosquito vector has remained unexplored. To address this, we offered *P. berghei*-infected *A. stephensi* additional naïve blood meals at different times relative to the infectious meal and assessed parasite prevalence and intensity compared to females that received only the infectious meal. An additional blood meal nearly doubled the number of sporozoites in mosquito salivary glands, but only when ingested by females with oocysts in early development. *P. berghei* infection did not affect mosquito fecundity or longevity, but downregulated key genes regulating egg production, suggesting malaria infection modulates resource allocation to oogenesis. These results demonstrate that iterative blood-feeding can increase the reproductive output of both *Plasmodium* and its vector, but timing of a second blood meal is critical for influencing *Plasmodium* fitness.

P34. Investigating Notch signaling in *Hymenolepis diminuta* segmentation

Chandler Lowe & Tania Rozario
UGA Department of Genetics & Center for Tropical and Emerging Global Diseases

Tapeworms cause significant damage to humans either directly by infection (e.g. cysticercosis or intestinal residence), or indirectly by impacting livestock health. The rat tapeworm, *Hymenolepis diminuta,* has recently re-emerged as a tractable model organism to study tapeworms. This tapeworm's body consists of a head with attachment organs, a regeneration competent neck, and a strobilated body made of reproductive segments (proglottids). The tapeworm has remarkable growth and regeneration capabilities, attributed to adult stem cell (SC) populations. These SCs are steadily organized into proglottids, but what developmental queues regulate this process are unknown. An unbiased RNA sequencing analysis of the neck revealed both a *delta* and *notch* homolog posteriorly biased in expression. *In situ* hybridization of these transcripts revealed a cryptic structure that seems to prelude obvious segment formation (dubbed the signaling quartet). Since Notch signaling is known to play roles in segmentation in many bilaterians, we are investigating Notch signaling as a regulator of segmentation in *H. diminuta*. We find that all core components of this signaling pathway are conserved in the tapeworm, and that Notch signaling genes are expressed in patterns that suggest roles in segmentation. Additionally, preliminary gene knockdown studies of *notch-2* and *notch-4* produce two distinctly different phenotypes related to segment regeneration. Future experiments include expression and functional characterization of the signaling quartet, functional validation of the canonical Notch signaling pathway in *H. diminuta*, and exploring crosstalk between Notch signaling and other developmental pathways such as Wnt, Hedgehog, and FGF signaling. These studies will yield insights into how the unique deployment of conserved developmental pathways has allowed this obligate parasite to evolve its specialized body plan.

P35. Induced in vitro sexual commitment of *Plasmodium cynomolgi*

James Oristian[1,2], Dennis E. Kyle[1,2]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, 30601, [2]Dept of Infectious Diseases, University of Georgia, Athena, GA, 30601

Plasmodium vivax is the most geographically widespread malaria species, yet our understanding of its unique biology has been hindered by a lack of *in-vitro* culture systems and access to human clinical samples. Phylogenetically related species such as *P. cynomolgi* have been historically utilized in non-human primate models to further our understanding of *P. vivax* biology. Recently, *P. cynomolgi* was successfully adapted to long-term *in vitro* culture, expanding the utility of this model species. Gametocytes, sexually committed parasites capable of mosquito infection, are understudied in malaria spp. and are critical for life stage progression, making them an ideal target for *in vitro* study. Previous reports indicate overexpression of gametocyte associated genes Api-AP2G (AP2-G) and Gametocyte Development protein 1 (GDV1) in *P. falciparum* and *P. berghei* leads to massive *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 *P. cynomolgi*. We aim to utilize both centromere-containing episomal plasmids as well as CRISPR/Cas9 to achieve our goals. *P. cynomolgi* mutants capable of *in vitro* sexual commitment will be tested for mosquito infectivity via standard membrane feeding assay. This work will increase our understanding of sexual commitment in *P. vivax*- like parasites and create a valuable tool for future studies.

P36. Investigating antigen diversification of *Trypanosoma cruzi* within a single-host infection

Aylla von Ermland[1] and Rick L. Tarleton[1]
[1] Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA.

Chagas disease is caused by the persistence of the parasite *Trypanosoma cruzi*, which may resist immune clearance through simultaneous expression of multiple variants of surface and secreted proteins encoded by multigene families (MGFs), including trans-sialidases (TS), mucin and mucin-associated proteins. The high copy number –hundreds to thousands of variants– and the variation between *T. cruzi* isolates suggest a system under intense pressure for diversification, shown to be driven by recombinational events of gene conversion and duplication. However, it remains unknown if these events accumulate gradually over evolutionary time or occur more rapidly, such as during a single host infection. To address this, we infected mice with a *T. cruzi* clone and recovered parasites ~1-year post-infection. Using whole-genome nanopore sequencing, reads aligned to the reference genome revealed high variability within MGF regions in all post-infection populations which was not present in the initial clone. While recombination and structural variant detection tools like RDP4 and NanoVar identified numerous recombination events enriched within MGFs, these methods were primarily designed for mammalian systems, limiting their performance in non-model organisms like *T.cruzi*. To overcome this limitation, we developed a pipeline to quantify new variants by aligning reads to the reference genome, converting their nucleotide variation signal into binary vectors and clustering them with DBSCAN to call gene variants. Analysis of one post-infection population (20x) revealed over 100 unique variants across the TS family, demonstrating active TS diversification during infection. Future studies will validate these events by sequencing post-infection clones and investigate diversification in earlier infection (~3-mo). This work contributes to the understanding of the pathogenesis of Chagas disease and the genetic diversity of parasite populations to which human hosts are exposed.

P37. Identification of β-carboline derivatives active against quiescent artemisinin-resistant *Plasmodium falciparum*

Lyric Wardlaw[1] Reagan Haney[1,4], Jopaul Mathew[5,6], Joshua H. Butler[1,4], Emily Bremers[1,4], Emilio F. Merino[1,4], Victoria Mendiola[2,4], Dennis Kyle[2,3,4], Maxim Totrov[9], Paul R. Carlier[7,8], Maria B. Cassera[1,4]

[1]Dept. of Biochemistry and Molecular Biology, [2]Dept. of Infectious Diseases, [3]Dept. of Cellular Biology, [4]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, USA; [5]Dept. of Chemistry [6]Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, Virginia, USA; [7]Dept. of Pharmaceutical Sciences [8]Dept. of Chemistry, University of Illinois at Chicago, Chicago, Illinois, USA; [9]Molsoft LLC, San Diego, California, USA.

Malaria is a life-threatening disease that caused approximately 263 million cases and 597,000 deaths in 2023. Declining clinical activity and resistance are affecting almost all currently used antimalarial drug classes, including artemisinin-based combination therapies (ACTs). Antimalarial resistance calls for the development of new chemotherapeutic agents with novel targets within the malaria parasite. In response, our research team has identified PRC1584, a promising lead antimalarial compound. Dihydroartemisinin (DHA) is an existing antimalarial commonly used in ACTs and it is known to induce a quiescent, or temporarily inactive, state in *Plasmodium falciparum* ring-stage parasites. This survival strategy increases the likelihood of treatment failure and can aid in the development of resistance. Understanding whether PRC1584 induces quiescence or terminates proliferating ring stages, and whether it is active against DHA-induced quiescent parasites in both resistant and sensitive strains is crucial. In this study, we investigated the activity of PRC1584 using the recrudescence assay (RA) and the Quiescent Survival Assay (QSA). Our results demonstrate that short-term exposure to PRC1584 effectively delays the recrudescence of DHA-resistant parasites compared to DHA-sensitive parasites in both the proliferating and DHA-induced ring stages. These findings reveal that PRC1584 is a critical candidate for overcoming the challenges of drug resistance in malaria therapy.

P38. Understanding the role of *nanos* in germ cell development and regeneration in *Hymenolepis diminuta*

Cierra Gladfelter[1,2] and Tania Rozario[1,2]

[1]CTEGD, UGA, Athens, GA [2]Department of Genetics, UGA, Athens, GA

Tapeworms are parasitic flatworms that cause disease worldwide in humans and livestock. Tapeworms exhibit significant regenerative capacity originating from their germinative neck region (GR), and this is likely where commitment to a germ cell fate occurs. As the regulation of germ cells is currently unknown, our research aims to molecularly characterize germ cell development in the rat tapeworm, *Hymenolepis diminuta*, by uncovering genes necessary for growth and reproduction. *Nanos*, a conserved germ cell regulator, is expressed in both germ and somatic stem cell populations in other worms like schistosomes and acoels. We performed whole mount in situ hybridization (ISH) and found *nanos* expression throughout the GR, genital anlagen, and gonads in tapeworms. To ascertain if *nanos+* cells in the GR bear hallmarks of germ cells, we performed double fluorescent ISH with other putative germ cell markers. All gonadal markers examined co-express with *nanos* in the GR, suggesting *nanos* expression marks early germ cells. Interestingly, one gene (*protocadherinα*) expresses in a subset of *nanos+* cells indicating heterogeneity within the *nanos+* population. The co-expression is confined to the most anterior part of the neck, where regenerative ability is most pronounced. Silencing of *nanos* using RNA interference (RNAi) shows a depletion in the total number of pre gonadal germ cells (PGGCs) in the GR, indicating it is functionally important for maintaining the PGGCs. Future *nanos* RNAi experiments will be conducted to determine its role in maintaining gonadal structures and the propensity of the tapeworm to regenerate. Our study will resolve the role(s) of *nanos* and help demystify how germ cells influence tapeworm regeneration.

P39. Understanding how *T. cruzi* infection is controlled in muscle

Jose Saenz[1], Fernando Sanchez[1], Jihyun Lim[1] and Rick Tarleton[1]
[1]CTEGD, UGA, Athens, GA

The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, a neglected tropical disease that can result in severe cardiac complications. The early infection period with *T. cruzi* may be accompanied by a high parasite burden, and the infection and replication within of a wide variety of host cell types. The generation of a robust humoral and cellular immune response controls parasite burden in most hosts but fails to completely resolve the infection. The resulting chronic infection is marked by the persistent presence of *T. cruzi*, including intracellularly primarily in muscle. The basis of the transition from infection of a broad array of host cell types to a restriction primarily to muscle cells is not known but is heavily dependent on the production of interferon gamma (IFN-γ) by *T. cruzi*-specific T cells. To specifically investigate the role of muscle in parasite control, we utilized a conditional transgenic mouse model in which the interferon-gamma receptor (IFNGR) is selectively knocked out in skeletal muscle tissue. Deletion of IFNGR in myocytes resulted in a significant increase in parasite burden within skeletal muscle, suggesting that IFN-γ -stimulated myocytes actively contribute to the containment of *T. cruzi* infection and/or replication. To further explore this role, differentiated myotubes were exposed to IFN-γ in vitro, exhibiting a robust transcriptional response. However, when myotubes were pre-treated with IFN-γ prior to infection with *T. cruzi*, not significant difference in infection rates were observed compared to untreated controls. Additionally, a mouse model of some of the most up-regulated effectors (Ch.3 GBP -/-) did not show difference in parasite burden comparing to WT mice. These findings suggest that IFN-γ alone is insufficient to confer resistance in myocytes at the conditions tested and that additional immune or paracrine factors may be required for effective parasite control. Further in vivo and in vitro studies are needed.

P40. Investigating the role of putative membrane contact site proteins in *Toxoplasma gondii*

Melissa Rogers[1, 2] Fabien Alpy[3], and Diego Huet[1, 2].
[1]CTEGD, UGA [2]Department of Cellular Biology, UGA [3]Institute of Genetics and Molecular and Cellular Biology (IGBMC), UMR 7104, CNRS, Inserm, Strasbourg University, Illkirch, France.

Membrane contact sites (MCSs) are regions of the cell in which two organelles are close enough together to be connected by tethering proteins without their membranes being fused. MCSs in yeast and mammals facilitate inter-organellar lipid and calcium transport. In the apicomplexan parasite *Toxoplasma gondii*, little is known about the proteins involved in MCS formation or what roles MCSs play in the parasite. In this study, we investigate the role of the START-like domain containing protein (TgSTART) and the WD domain, G beta repeat-containing protein (WDR44) in MCS formation and function in *T. gondii.* TgSTART was identified as a protein of interest during a bioinformatic search for proteins containing lipid transfer domains (START domains) in *T. gondii*. In mammals, START domain-containing proteins have been implicated in MCS-mediated lipid transport. WDR44 was identified during a search for proteins containing FFAT domains in *T. gondii*. In mammals, FFAT domain-containing proteins bind the protein VAP on the surface of the endoplasmic reticulum and can serve as MCS intermediates. Due to the presence of a predicted START domain in TgSTART and a predicted FFAT domain in WDR44, we hypothesize that these proteins could mediate MCSs in *T. gondii*. Here we show the strategies used to conditionally knock down protein expression of TgSTART and WDR44 in *T. gondii* to further explore the role of these proteins. We investigate the integration of the conditional knockdown system into *T. gondii* parental strains using immunofluorescence assays, PCR, and western blotting. Future studies will use these conditional knockdown strains to assess the role of TgSTART and WDR44 in lipid transport at MCSs as well as the overall growth and replication of the parasite.

P41. VEuPathDB: Tools for genomic-scale data exploration, analysis, integration and discovery

Nupur Kittur[1], Omar S. Harb[2] & David S. Roos[2] … on behalf of the entire VEuPathDB team
[1]Center for Tropical and Emerging Global Diseases, UGA, Athens, GA USA [2]Department of Biology, U Penn, Philadelphia, PA USA

Biomedical research is increasingly driven by Big Data, from genome sequencing to population-level diversity and multi-Omics datasets. How can we effectively collect, store, maintain and integrate this information to ensure FAIR (**F**indable, **A**ccessible, **I**nteroperable, **R**eusable) data access, advancing biological understanding and defining targets for further study in the lab, field & clinic? The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org, including PlasmoDB.org, ToxoDB.org, CryptoDB.org, VectorBase.org and other platforms) is a free, scalable data resource serving thousands of users daily. It enables discovery in eukaryotic pathogen and vector research without requiring advanced computational skills. Key features include access to diverse datasets such as genomes, transcriptomes, proteomes, motifs, pathways, and more, tools for comparative genomics, epigenetics, and orthology-based function prediction, integration and in silico analysis of public and user datasets, annotation improvement and community curation, and compliance support for funder and publisher data policies. Recent features include alpha-fold structures, long-read & single-cell RNAseq data and improved orthology detection. Upcoming features include AI-based literature curation and summaries of expression data.

P42. Membrane contact site assembly is required for VDAC-dependent mitochondrial calcium uptake in *Toxoplasma gondii*

Abdul Malik Hussein[1], Myriam Andrea Hortua-Triana[1], Silvia N J Moreno[1]
[1]CTEGD, Department of Cellular Biology, UGA, Athens, Georgia, USA

In *Toxoplasma gondii*, as in other eukaryotes, calcium homeostasis is essential for cell survival. This balance is maintained by actively transporting excess cytosolic calcium into the endoplasmic reticulum (ER) via the SERCA pump, and by extruding calcium out of the cell through a plasma membrane calcium ATPase. In mammalian cells, mitochondria contribute to the cytosolic calcium homeostasis by taking up calcium through a voltage-dependent anion channel (VDAC) at the outer mitochondrial membrane, and the mitochondrial calcium uniporter at the inner mitochondrial membrane. VDAC is localized at ER-mitochondria membrane contact sites (MCS) where it permits the direct transfer of calcium from the ER to the mitochondria. In yeast and other organisms, the mitochondria is coupled to the ER via a tripartite protein complex, VDAC-GRP75-IP_3R. This complex specifically allows the ER to release calcium through the IP_3R resulting in accumulation of high concentration of localized calcium between the organelles known as calcium microdomains, which license the mitochondrial calcium uptake through VDAC at the contact sites. While MCS is well described in other systems, its existence and function in *T. gondii* remain largely unexplored. Notably, orthologs of VDAC and GRP75 are present in *T. gondii*, but their roles in calcium dynamics are unclear. Here, we show that *T. gondii* VDAC, when heterologously expressed in HeLa and HEK cells, lead to reduced cytosolic calcium increase upon histamine or carbachol stimulation, pointing to an enhanced mitochondrial calcium uptake. This result suggests that *T. gondii* VDAC is a functional calcium channel that becomes active at ER–mitochondria contact sites. We also showed that knocking down GRP75 in *T. gondii* leads to elevated cytosolic calcium and significantly reduced mitochondrial calcium uptake. Co-immunoprecipitation confirms a physical interaction between VDAC and GRP75. Together, these findings support a model in which *T. gondii* VDAC facilitates mitochondrial calcium uptake specifically through a GRP75-dependent membrane contact site with the ER.

P43. Preliminary characterization of two *Trypanosoma cruzi* isolates from northern Florida, U.S., suggests the potential for human infection

Ruby E. Harrison[1], Norman Beatty[2], Rodrigo de Paula Baptista[3], Kevin J. Vogel[4], Sandra Y. Mendiola[4], R. Drew Etheridge[1]

[1]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA [2]Division of Infectious Diseases and Global Medicine, University of Florida College of Medicine, Gainesville, FL [3]Houston Methodist Research Institute, Houston, TX [4]Dept of Entomology, University of Georgia, Athens, GA

Trypanosoma cruzi, a eukaryotic parasite that alternates between insect and mammalian hosts, causes Chagas Disease in humans which can progress to a debilitating chronic infection and potentially fatal cardiomyopathy. Infectious stages of *T. cruzi* are transmitted through contact with the excrement of its insect vector, the kissing bug (triatomine). Chagas Disease primarily occurs in Central and South America; while *T. cruzi* is commonly detected in kissing bugs and sylvatic animal reservoirs in North America, autochthonous transmission to humans is very seldom reported in this region. Potential explanations for this discrepancy include decreased frequency of human-vector contact or reduced virulence of North American *T. cruzi* strains. To examine the latter possibility, we developed a novel protocol to isolate *T. cruzi* from infected kissing bugs for axenic cultivation *in vitro*. We applied this method to obtain two new *T. cruzi* isolates from the North American kissing bug species *Triatoma sanguisuga* captured from a domicile near Gainesville, FL. Laboratory assays indicate that both isolates produce high numbers of mammalian-infectious stages *in vitro,* readily infect mouse cardiomyocytes*,* and colonize the model kissing bug *Rhodnius prolixus*. Strain typing of the two isolates placed them in the discrete typing unit (DTU) I lineage and whole genome sequencing of one isolate showed reduced overall size but high conservation compared to the model *T. cruzi* Y strain of South American origin. Genetic manipulation of the sequenced strain using an expression vector tailored for the Y strain was also successful. Together, these results indicate the infection potential of Floridian *T. cruzi* is on par with that of known pathogenic Central and South American strains. Hence, further epidemiological assessment is warranted to elucidate why autochthonous Chagas is so rarely reported in the United States.

P44. Tb927.8.2820, a target of NEU-4438, is important for endocytosis of transferrin and cell shape maintenance in *Trypanosoma brucei*

Gaurav Kumar[1], and Kojo Mensa-Wilmot[1,2]

[1]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA, [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA

Trypanosoma brucei is a protozoan parasite that causes Human African trypanosomiasis (HAT). Due to limitations in current therapies, we are developing new anti-T. brucei lead compounds, exemplified by a quinolinimine NEU-4438. Due to the use of whole trypanosome toxicity as a readout during its chemical optimization, molecular targets of NEU-4438 are not known. Employing photoaffinity labelling we identified hypothetical protein Tb927.8.2820 as an NEU-4438 binding protein. Tb927.8.2820 protein has a predicted C2 domain and contains a region of 33 PolyQP repeats, and it localized to the posterior tip trypanosomes. Genetic knockdown of Tb927.8.2820 showed the protein to be essential for survival of *T. brucei*. Depletion of the protein (12 h) prevented endocytosis of transferrin and caused rounding of cells. We conclude that Tb927.8.2820 has important functions in *T. brucei* consistent with its discovery as a target of NEU-4438.

P45. A SNARE-like *Plasmodium* rhoptry neck protein is required for sealing of the parasitophorous vacuole during merozoite invasion

Grace W. Vick[1,2], Carrie F. Brooks[1,3], Vincent Starai[4], Vasant Muralidharan[1,2,3]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, [2]Department of Infectious Diseases, University of Georgia, Athens, GA, [3]Department of Cell Biology, University of Georgia, Athens, GA, [4]Department of Microbiology, University of Georgia, Athens, GA

Plasmodium falciparum, the causative agent of human malaria, are obligate intracellular parasites during all replicative stages of their complex lifecycle. Critical for their successful expansion is their ability to form and replicate inside a vacuole derived from the host RBC membrane during invasion called the parasitophorous vacuole (PV). The molecular mechanisms that seal the PV from the RBC membrane during merozoite invasion are not well understood. Our work has identified a conserved membrane protein (Pf3D7_1117400) that has distant homology to vesicle-SNARE proteins. Using CRISPR/Cas9 genetic engineering to create a conditional knockdown of Pf3D7_1117400, we determined that it localizes to the rhoptry neck. Hence, we termed this protein, SNARE-like rhoptry neck protein (SNARON). Knockdown of SNARON led to parasite death, indicating that SNARON is essential for intraerythrocytic development. We observe that SNARON knockdown parasites egress normally, but fail to form rings. Using live video microscopy, we show that SNARON-deficient parasites are able to perform all steps of invasion prior to PV formation, but fail to successfully internalize into host cells. When SNARON is expressed in *Saccharomyces cerevisiae*, we find that vacuolar membranes form defective structures, indicating a role for SNARON in membrane fusion. Together, these data suggest that SNARON works at the site of merozoite invasion to drive sealing of the PV from the RBC membrane.

P46. Tick Genomes: Overcoming the limitations of tick biology with advancements in sequencing technology

Katie C. Dillon[1], Julia C. Frederick[2], Isobel Ronai[3,4], Travis C. Glenn[1,2]
[1]Institute of Bioinformatics, University of Georgia, Athens, GA, USA, [2]Department of Environmental Health Science, University of Georgia, Athens, GA, USA, [3]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA, [4]Howard Hughes Medical Institute, Chevy Chase, Maryland 20815, USA

More than 900 species of ticks have been described, however, genome assemblies are available for less than 3% of these species. Major challenges for generating genome assemblies of ticks are due to their unique biology, including: small body size, large genome size, DNA contamination, and a high proportion of novel transposable elements. We summarize how developments in genome sequencing and assembly software have dramatically improved tick assemblies from 2008-2024. Recent advances in genome sequencing technologies have led to an increasing number of tick genomes, often based on sequences from single individuals, and enabled the generation of high-quality tick genome assemblies. We use a consistent set of bioinformatic approaches to characterize and assess the 50 genome assemblies that are publicly available for 23 tick species within the National Center for Biotechnology Information (NCBI) database as of February 2025. By directly comparing the assemblies, we identify 35 high quality assemblies, which should be used in downstream analyses. These high-quality assemblies represent 17 species of ticks. In addition, even though most are highly fragmented, we find 25 assemblies have more than 90% of the expected conserved genes. Finally, we discuss ongoing challenges and future directions for tick genome sequencing and assembly. Tick genome assemblies are critical biological resources that are leveraged to advance tick research, including a better understanding of the molecular basis of tick-host-pathogen interactions and pesticide resistance, alongside identifying potential vaccine targets.

P47. Immunogenicity of a protein nanoparticle vaccine encoding the *Plasmodium falciparum* MIF protein in *Aotus nancymaae*

Amadis Vivas[1,2], Celia L. Saney[1,2], Sarah Gayle Roberson[1,2], Ashley Gooch[1,2], Gina Kim[4], Cara Chao [5], Mark Langowski[5], Neil King [5], Richard Bucala [6], Chester J. Joyner[1,2,3]

[1]CVI, UGA, Athens, GA [2]CTEGD, UGA, Athens, GA [3]Department of Infectious Disease, UGA, Athens, GA [4]University Research Animal Resources, UGA, Athens, GA [5]Department of Biochemistry, University of Washington, Seattle, WA [6]Yale University School of Medicine, New Haven, CT

Malaria parasites encode a MIF ortholog, *Plasmodium* MIF (pMIF), that interferes with host MIF signaling during infection. In rodent malaria models, pMIF drives a pro-inflammatory environment which impairs Tfh cell differentiation, leading to reduced germinal center activity and anti- *Plasmodium* antibody production. When vaccinated against pMIF, these responses are improved and lead to improved parasite control in a subsequent challenge. These data suggest that immunization with pMIF may enable the development of malarial immunity rapidly after natural exposures, and therefore, reduce malaria morbidity and mortality. However, it remains unclear whether immunization with pMIF from *Plasmodium falciparum* (*Pf*) will lead to the same immunological outcomes observed in rodent malaria models. Here, we assessed the immunogenicity of a protein nanoparticle vaccine encoding *Pf*MIF in *Aotus nancymaae*, the standard preclinical model for evaluating vaccines against *Pf*. We show that the *Pf*MIF nanoparticle vaccine with AddaVax is immunogenic and induces reciprocal anti-*Pf*MIF IgG titers of > 2,430 by 28 days post single dose vaccination. No adverse events were observed. Together, this study demonstrates that a *Pf*MIF nanoparticle vaccine adjuvanted with AddaVax is immunogenic in *Aotus* monkeys and sets the stage for testing the efficacy of *Pf*MIF vaccination in this model.

P48. Establishment of transfection approaches in *Naegleria fowleri*

Caroline Palmentiero, Colm Roster, Jillian McKeon, and James Morris

Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry

Clemson University, Clemson, SC

The pathogenic free-living amoeba, *Naegleria fowleri*, is the causative agent of primary amoebic meningoencephalitis (PAM). This infection has a fatality rate of 97% in part due to the lack of readily available and effective drugs. The development of molecular tools to enable trans-gene expression in this organism will provide vital insight into gene function while allowing genetic validation of potential drug targets. We have developed an expression vector, pCJ2, using UTRs of conserved actin and ubiquitin genes from the *N. fowleri* TY strain (the reference genome) to drive expression of the puromycin N-acetyltransferase (PAC) resistance gene and the enhanced yellow fluorescent protein (eYFP) gene respectively. For transfection, we used polyethylenimine (PEI) cationic polymers, which bind to the negatively charged phosphates in DNA, to form complex nanoparticles that enhance plasmid uptake. Through electrophoretic mobility shift assay, we determine an optimal plasmid:PEI ratio of 1:1 (by weight) for a 40 KDa PEI (PEI-40). We have observed eYFP expression in a portion of cells transfected with pCJ2, but this fluorescence was transient and only detectable for up seven days. Moreover, puromycin drug treatment ultimately killed all the transfected cells. Therefore, we developed a second expression vector containing codon optimized PAC flanked by the actin UTRs and have generated stable antibiotic-resistant cell lines. Overall, we have established a technique for transgene integration into cells through PEI-40, and transgene expression driven by both actin and ubiquitin UTRs. Future work is aimed at optimizing our plasmids to enhance stable expression of both PAC and various fluorescent markers. With these tools, we anticipate being able to ask fundamental questions about amoeba biology and to validate drug targets.

P49. Differentially expressed genes in the in vitro activation of *Taenia solium* larvae by taurocholic acid

David Castaneda-Carpio[1], Segundo Del Aguila[1,2], Jose Maravi-Jaime[1], Renzo Gutierrez-Loli[1,3], Valeria Villar-Davila[1], Cristina Guerra-Giraldez[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru. [2]Wistar Institute, Philadelphia, Pennsylvania, USA. [3]Université Paul Sabatier, Toulouse, France

The parasitic flatworm *Taenia solium* grows as an egg-producing adult tapeworm inside the human intestine; this happens after bile acids and digestive enzymes trigger the evagination of the scolex from the larval cyst, allowing attachment to the lining of the gut. We employed Illumina paired-end RNA sequencing to examine differentially expressed genes (DEGs) and their associated biological processes in the presence and absence of taurocholic acid (TA), a bile component, in seven in vitro conditions: non-cultured parasites (NC); non-evaginated (PRE) parasites, cultured for 6 h with TA (TA+) or without TA (TA-); recently-evaginated (EV) parasites, cultured up to 2 days with TA+ or TA-; and fully evaginated (POST) parasites, cultured for 5 days with TA+ or TA-. Comparing EV and PRE, 309 DEGs were upregulated (UR) and 398 downregulated (DR) exclusively in TA+ conditions, while 144 DEGs were UR and 88 DEGs were DR in TA- conditions. Other DEGs were common to TA+ and TA- conditions. Exploration by GSEA showed that processes associated with "Monatomic ion transport" and "Intracellular signal transduction" were more active before evagination, and "Transcription by RNA polymerase II", "mRNA splicing via spliceosome", "Translation" and "Protein folding" were enriched after evagination of the scolex. Our findings provide insights into explaining how TA triggers *T. solium*´s development in vitro, as proxy to bile acids in vivo.