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Program

8:30 AM  Registration and Poster Set-Up
9:00 AM  Opening Remarks: Daniel Colley, CTEGD and Dept. of Microbiology, UGA
9:10 AM  Amy Styer Greene, CTEGD and DEPT. of Biochemistry & Molecular Biology, UGA
          The Mechanism of Apolipoprotein L1 Killing of Trypanosoma brucei brucei
9:30 AM  Anat Florentin, Center for Tropical & Emerging Global Diseases, UGA
          The Apicoplast Chaperone CLPC is Essential for Intra-Erythrocytic Growth of Plasmodium falciparum
9:50 AM  A. J. Stasic, CTEGD AND Dept. of Cellular Biology, UGA
          The Toxoplasma gondii Vacuolar-H+-ATPase
10:10 AM Break – Poster Viewing
10:50 AM Jessica Lopes da Rosa-Spieglar, CTEGD AND Dept. of Biochemistry & Molecular Biology, UGA
          Transcription Termination of a Polycistronic Genome
11:10 AM Sarah Bauer, Eukaryotic Pathogen Innovation Center, Clemson University
          Glucose-Dependent Dual Localization of the Glycosome Protein TbPEX13.1
11:30 AM Rodrigo Baptista, Center for Tropical & Emerging Global Diseases, UGA
          Genefamily Copy Number Variation Throughout the Cryptosporidia
11:50 AM Evgeniy Potapenko, CTEGD and Dept. of Cellular Biology, UGA
          Phosphate Transport by the Acidocalcisome Transporter TbPho91 of Trypanosoma brucei
12:10 PM Lunch – Poster Viewing
1:30 PM  Michael Schultz, Dept. of Microbiology, University of Alabama-Birmingham
          Targeting the NAD Salvage Pathway for the Treatment of Schistosomiasis
1:50 PM  Anthony Szempruch, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
          Extracellular Vesicles from Trypanosoma brucei Mediate Virulence Factor Transfer and Cause Host Anemia
2:10 PM  Ousman Mahmud, CTEGD and Dept. of Genetics, UGA
          Dissecting the Movement and Transport Roles of Apicomplexan Dyneins
2:30 PM  Break – Poster Viewing
3:15 PM  Duo Peng, Dept. of Cellular Biology, UGA
          Modification of a gRNA Design Webtool for CRISPR/Cas9-Based Whole Genome Screens in Trypanosoma cruzi and Other Eukaryotic Pathogens
3:35 PM  Angela Pack, CTEGD and Dept. of Microbiology, UGA
          Trypanosoma cruzi Specific CD8+ T Cells Continue to Mediate Parasite Control Despite Chronic Antigen Exposure
3:55 PM  Introduction of the Keynote Speaker
4:00 PM  John Boothroyd, Dept. Microbiology & Immunology, Stanford University School of Medicine
          Communicating Outside the Bubble: How the Intracellular Parasite, Toxoplasma gondii, Moves Proteins Across the Parasitophorous Vacuole
5:00 PM  Adjourn
Poster Presentations

P1. **Stephen Patrick**, Dept. of Genetics & Biochemistry, Clemson University
Protozoan Hexokinases as Therapeutic Targets: *Trypanosoma brucei* and *Plasmodium falciparum* in the Crosshairs

P2. **Susanne Warrenfeltz**, Center for Tropical & Emerging Global Diseases, UGA
EuPathDB: A Powerful Eukaryotic Pathogen Data Mining Resource with a Unique Search Strategy System

P3. **Dragan Ljolje**, Dept. of Parasitic Diseases and Malaria, CDC
Detection of *Plasmodium* Parasites with Loop Mediated Isothermal Amplification (LAMP) Using Simple Sample Preparation Methods

P4. **Pooya Dogra Saraf**, Dept. of Microbiology, University of Tennessee
The Origin and Transmission Pattern of Type II Dominant Lineage of *Toxoplasma gondii*

P5. **Jessica Aycock**, Dept. of Biological Sciences, Mississippi State University
A Survey of Mississippi Mosquitoes’ Blood Meal and Avian Malaria Parasites

P6. **Ruby Coates**, CTEGD and Dept. of Infectious Diseases, UGA
There are Complex Interactions Between Parasitic Helminths, Drug Treatments and the Host Innate Immune System

P7. **Yun He**, Dept. of Parasitic Diseases & Malaria, CDC
Towards the Development of Next Generation of Rapid Diagnostic Test: Synthesis of Glycophosphatidylinositol (GPI) Analogues of *Plasmodium falciparum* and Immunological Characterization

P8. **Mary Maclean**, CTEGD and Dept. of Infectious Diseases, UGA
In vivo Effects of Drugs Used in Lymphatic Filariasis MDA Programs on *Brugia malayi* in Gerbils

P9. **Whitney Bullard**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
From Parasitic Protozoa to Stem Cells and Beyond: The Implications of a Trypanosome Glucosyltransferase in the Field of Mammalian Epigenetics

P10. **Cybelle Tabillas**, Dept. of Genetics, UGA
Investigating the Role of the Notch Signaling Pathway in the Development of Thymic Epithelial Cells

P11. **Ciro Cordeiro**, CTEGD and Dept. of Cellular Biology, UGA
Identification of New Family of Inorganic Polyphosphate Polyphosphatases in *Trypanosoma brucei*

P12. **Heather Bishop**, CTEGD and Dept. of Cellular Biology, UGA
*Plasmodium falciparum* ER Resident Protein, PfGRP170, is Required for Asexual Growth and Gametocyte Formation

P13. **Phil Yao**, CTEGD and Dept. of Cellular Biology, UGA
Enhancing Host Immune Response to *Trypanosoma cruzi* Through Expression of PAMPs and DAMPs

P14. **Michael Mills**, Dept. of Microbiology, UGA
Wolbachia Protein Wbmo076 Modulates Yeast Actin Dynamics

P15. **Catherine Smith**, CTEGD and Dept. of Infectious Diseases, UGA
Development of a Mouse Model for Pregnancy Maintenance During Maternal Malaria Infection

P16. **Raquel Silva de Negreiros**, CTEGD and Dept. of Cellular Biology, UGA
Investigation of the Chaperone Activity of Polyphosphate in Trypanosomes

P17. **Alexis Thomas**, Dept. of Infectious Diseases, UGA
Enrichment of Human Spermatogonia via Magnetic Activated Cell Sorting and Optimization of Cryopreservation

P18. **Kristen Fowler**, Dept. of Genetics & Biochemistry, Clemson University
Do Acyl-CoA Synthetases Mediate Fatty Acid Uptake in *T. brucei*?

P19. **David Reynolds**, Dept. of Biochemistry & Molecular Biology, UGA
Base J Represses Genes at the End of Polycistronic Gene Clusters in *Leishmania major* by Promoting RNAP II Termination

P20. **David McKinney**, CTEGD and Dept. of Entomology, UGA
Cholesterol Localization and Ecdysteroidogenesis in the Mosquito *Aedes aegypti*
P21. **Nicole-Lisa Williams**, CTEGD and Dept. of Infectious Diseases, UGA
Prior Exposure Seasonal H1N1 Influenza Shapes Specificity of Pandemic H1N1 Influenza Antibody Responses and Virus Evolution

P22. **Tankya Simoneaux**, Dept. of Microbiology, Biochemistry & Immunology, Morehouse School of Medicine
A Unique Insight into the MiRNA Profile During Genital Chlamydial Infection

P23. **Erica Burkman**, Dept. of Infectious Diseases, UGA
Investigating Early Infection Status of the Filarial Parasite *Brugia malayi* in the Cat, the Laboratory Model for Lymphatic Filariasis

P24. **Justin Fellows**, CTEGD and Dept. of Cellular Biology, UGA
A Central Role for the Ubiquitin-Like Protein Apiquitin in Apicoplast Protein Import in *T. gondii*

P25. **Ivelisse Resto-Garay**, Dept. of Infectious Diseases, UGA
Generation of a Recombinant Nkp46 Protein to Evaluate Influenza Hemagglutinin (HA) Protein Binding to NK Cell Receptors

P26. **Jeffrey Eells**, Dept. of Basic Sciences, Mississippi State University
Elevated Dopamine Levels and Reduced Dopamine Turnover in the Striatum After Toxoplasma gondii Infection

P27. **Fernando Sánchez-Valdés**, Center for Tropical & Emerging Global Diseases, UGA
Differential Sensitivity to Benznidazole Among *Trypanosoma cruzi* Isolates is Associated with Isolate-Specific Tissue Tropism

P28. **Serena Walker**, Dept. of Genetics & Biochemistry, Clemson University
Characterization of Mode of Action of Hoechst Analogs on Bloodstream Form T. brucei

P29. **Paige Tehan**, Dept. of Biological Sciences, Clemson University
Understanding the Roles of Chloroquine Resistance Transporter Homolog (TgCRT) in the Pathogenesis of Toxoplasmosis

P30. **Miryam Hortua Triana**, Center for Tropical & Emerging Global Diseases, UGA
Two Mechanisms of Ca²⁺ Entry in *Toxoplasma gondii*

P31. **Emily Myers**, CTEGD and Dept. of Cellular Biology, UGA
Elucidating the Role of Trehalose-6-Phosphate Synthase in the Development and Transmission of *Cryptosporidium parvum*

P32. **Logan Crowe**, EPIC and Dept. of Genetics & Biochemistry, Clemson University
Identification and Characterization of a Pex3-Like Protein (TbP3L) in *T. brucei*

P33. **Myles Chetcutti**, EPIC and Dept. of Genetics & Biochemistry, Clemson University
Lipid Droplets in the Trypanosomatid *Crithidia fasciculata*

P34. **Mattie Pawlowic**, Center for Tropical & Emerging Global Diseases, UGA
Understanding Transport in *Cryptosporidium parvum*

P35. **Jeannie Stubblefield**, Dept. of Molecular Biosciences, Middle Tennessee State University
SAR-Guided Optimization of Anti-Trypanosomal Aurones

P36. **Rudo Kieft**, Dept. of Biochemistry & Molecular Biology, UGA
Regulation of Base J Synthesis in Kinetoplastids

P37. **Juliana Assis Geraldó**, Genomics & Computational Biology Group, CPqRR-FIOCRUZ
Exploring the Role of Small Non-Coding RNA During Zika Infection

P38. **Shelton Griffith**, CTEGD and Institute of Bioinformatics, UGA
Comparative Analysis of Illumina and Hybrid Assembly Annotations for *Cryptosporidium baileyi*

P39. **Manuel Pierro**, CTEGD and Dept. of Cellular Biology, UGA
Assessing the Role of PpErc in the Regulation of Calcium in *P. falciparum*

P40. **Jayesh Tandel**, CTEGD and Dept. of Cellular Biology, UGA
Understanding the Development of the Sexual Stages of *Cryptosporidium parvum*

P41. **Nathan Chasen**, CTEGD and Dept. of Infectious Diseases, UGA
Zinc Transporters and the Maintenance of Zinc Homeostasis During the *Toxoplasma gondii* Lytic Cycle

P42. **Shubham Basu**, CTEGD and Institute of Bioinformatics, UGA
Resolution of Genome Duplications in *Toxoplasma gondii*
Communicating Outside the Bubble: How the Intracellular Parasite, Toxoplasma gondii, Moves Proteins Across the Parasitophorous Vacuole

The major pathogenic species of Apicomplexa are all obligate intracellular parasites that generally reproduce within a parasitophorous vacuole (PV) found within the infected host cell. This niche presents both an opportunity and a challenge to the parasites growing within – on the one hand, they are sequestered from some of the immune sensors and defenses that might detect their presence but at the same time, the PV membrane (PVM) presents a physical barrier to export of protein effectors necessary to modulate host functions to the parasite’s advantage.

We use the ubiquitous Apicomplexan Toxoplasma gondii as a model for the study of intracellular parasitism. In this talk, I will describe how we started with an unusual ability of the parasite to induce the potent host oncogene, c-Myc, and then employed genetic strategies to identify the machinery involved in the export of the effector that drives this induction. The results reveal a novel collection of proteins that mediate the movement of dense granule proteins across the PVM where they can coopt myriad host pathways needed for parasite growth.
The Mechanism of Apolipoprotein L1 killing of *Trypanosoma brucei brucei*

Amy Styer Greene¹, Stephen Hajduk¹
CTEGD & Biochem. Mol. Biol., University of Georgia

*Trypanosoma brucei brucei* parasites are rapidly lysed by human serum due to innate immune factors called trypanosome lytic factors (TLFs). Related parasites *T. b. rhodesiense* and *T. b. gambiense* evolved resistance to the TLFs and cause the deadly disease human African trypanosomiasis. My work focused on TLF-1, a high density lipoprotein particle (HDL) containing the pore-forming toxin Apolipoprotein L1 (ApoL1) and hemo-globin-binding protein haptoglobin related protein (Hpr). I found that TLF-1 and ApoL1 cause rapid changes at the plasma membrane, including dissipating the plasma membrane potential and inducing sensitivity to hypotonic lysis. ApoL1-induced osmotic stress at the plasma membrane leads to water influx and eventual lysis. Moreover, osmotic lysis is exacerbated by oxidizing agents and molecules which bind to free thiols in proteins. My work suggests that ApoL1 in TLF-1 traffics to the plasma membrane of *T. b. brucei*, facilitating sodium influx, ionic imbalance, oxidation of osmoregulatory proteins, cell swelling, and trypanosome lysis.

The Apicoplast Chaperone Clpc Is Essential For Intra-Erythrocytic Growth of *Plasmodium falciparum*

Anat Florentin, Jillian Fishburn, Michelle Krakowiak, Paul Kim, Manuel Fierro and Vasant Muralidharan
CTEGD, University of Georgia

Like other apicomplexan parasites, Plasmodium falciparum contains a non-photosynthetic plastid known as the apicoplast. The functions of the apicoplast include several important metabolic pathways, involving enzymes that are encoded by the nuclear genome and then are somehow transported to the organelle. These proteins usually contain a bipartite apicoplast targeting sequence consisting of a signal peptide and a transit peptide but their mode of targeting, transport and the mechanism by which they cross the four membranes of the apicoplast are poorly understood. We hypothesized that the apicoplast-resident Clp family of chaperones and proteases are involved in protein trafficking to the apicoplast as well as the homeostasis of the organelle. To test that we used a conditional knockdown approach that allows us to inhibit function, determine localization and purify proteins with their partners. The gene fusion tag combines an inducible DHFR-based degradation domain (DDD) with HA-epitope. The DDD is stabilized in the presence of trimethoprim (TMP) and upon its removal the unfolded DDD binds the fused chaperone intramolecularly and inhibits protein function. We successfully tagged the ClpC ATPase and obtained 2 separate clones from 2 independent transfections. We found that under normal culturing conditions the tagged chaperone localizes to the apicoplast. Removal of the stabilizing agent, however, results in disruption of its apicoplast localization and the tagged protein accumulated instead in vesicle-like structures. Importantly, culturing of the transgenic parasites in the absence of TMP results in growth inhibition, indicating that ClpC is essential for parasite viability. Altogether, we found an essential gene with previously unknown function, potentially involved in apicoplast homeostasis and trafficking.
**The Toxoplasma gondii Vacuolar–H⁺-ATPase**

Andrew J. Stasic¹, Stephen A. Vella¹, Vincent Starai² and Silvia N.J. Moreno¹

¹Center for Tropical and Emerging Global Diseases, University of Georgia, Athens GA, 30602, ²Department of Microbiology, University of Georgia, Athens GA, 30602

*Toxoplasma gondii* is an obligate intracellular parasite of great medical significance. The rapidly growing tachyzoite enters host cells by a process of active invasion, replicates inside the cell and, after a certain number of replications exits to infect neighboring cells. While searching for a host cell, the parasite is exposed to an environment with an ionic composition quite different from the host intracellular composition. This sharp change from intracellular to extracellular milieu poses a challenge to the fitness of the parasite, which needs to glide, extrude its conoid and secrete proteins from apical secretory organelles (micronemes and rhoptries) to form the moving junction and the parasitophorous vacuole for its next intracellular life. *T. gondii* possesses a lysosomal compartment termed plant-like vacuole or PLV that appears to be involved in ionic homeostasis (similar to the plant vacuole). Proteomic analysis of an enriched PLV fraction showed several subunits of the vacuolar-H⁺-ATPase (V-H⁺-ATPase), an evolutionarily conserved proton pumping complex. We inserted a hemagglutinin (HA) tag at the endogenous locus for the *T. gondii* a₁, E, and G subunit genes of the *T. gondii* TgVHA1. IFA analysis showed that the a₁ subunit (TgVHA1) localizes to the plasma membrane and the plant-like vacuole (PLV). A time course analysis of recently egressed parasites by “high resolution” microscopy and 3D reconstruction showed that TgVHA1 forms a large punctum and a row of vesicles connecting the plasma membrane and the PLV. We have created a TgVHA1 conditional knockout strain, which shows a significant growth defect. Ablation of TgVHA1 resulted in reduced motility, egress, microneme secretion, and invasion/attachment. The defect in invasion is likely due to reduction in the secretion of the adhesin TgMIC2. We also observed abnormal localization of microneme proteins. Biochemical function of TgVHA1 was studied using yeast mutants deficient in both yeast a subunits.

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**Transcription Termination of a Polycistronic Genome**

Jessica Lopes da Rosa-Spiegler, Kieft, R. & Sabatini, R.

BCMB, CTEGD, UGA

In typical Eukaryotes, transcription of a single gene initiates at a defined promoter region and terminates at a distinct transcription termination site (TTS) delineated by RNA processing signals. However, in Kinetoplasts, genes are clustered in long directional arrays called polycistronic transcriptional units (PTUs), with a single upstream promoter region for the PTU. Aside from epigenetic factors unique to Kinetoplastids, it is not known how the polymerase transcribes continuously across multiple genes and processing signals and dissociates specifically at the end of PTUs.

The CPF-CF pathway is the dominant mode of transcription termination in higher Eukaryotes. A ribo-endonuclease cleaves nascent RNA at the poly-adenylation site (pAS), which allows for subsequent processing and stabilization of RNA. The remaining nascent RNA, which is partially associated with DNA and the polymerase, is degraded by a 5’-3’ ribo-exonuclease. In certain cases, an RNA-DNA helicase promotes degradation of nascent RNA. Ultimately, RNA polymerase II is destabilized from the template via what is called the “torpedo”-allosteric model.

We show that although various components of the “torpedo”-allosteric model exist in *T. brucei*, their function as it pertains to termination is distinct from nascent RNA processing. We identified the pAS-specific ribo-endonuclease of the CPF-CF complex. We show that it associates with expected CPF-CF sub-units and that it is required to cleave nascent PTU RNA into mono-cistronic transcripts. However, our data shows that pAS-specific cleavage is not involved in termination. Accordingly, our data shows that nascent RNA degradation by the 5’-3’ ribo-exonuclease is also not required for termination. We conclude that unlike higher Eukaryotes *T. brucei* does not couple nascent RNA cleavage to transcription termination. Interestingly, we found that a putative homologue of the aforementioned RNA-DNA helicase and a Trypanosome-unique RNA-binding protein are involved in transcription termination.
Glucose-Dependent Dual Localization of the Glycosome Protein TbPEX13.1

Sarah Bauer, Terral Patel, Kelley McQueeney, Meredith Teilhet Morris
Eukaryotic Pathogens Innovation Center, Clemson University

Glucose levels fluctuate during the lifecycle of Trypanosoma brucei and have a profound effect on parasite morphology and metabolism. These changes include the up-regulation of enzyme activities in the glycolytic pathway, which is compartmentalized within highly-specialized peroxisomes named glycosomes. These organelles are essential to parasite survival and any disruption of their integrity is lethal. While protein import into these organelles and glucose-dependent regulation of enzyme activities have been studied, the mechanisms that govern their biogenesis, proliferation, and environmentally regulated changes in protein composition are unknown. We are utilizing a number of biochemical and microscopic approaches to define the molecular composition of glycosomes under different glucose levels. Glycosome protein levels and sensitivity to the detergent digitonin, which is used to assess glycosome localization, change in response to extracellular glucose levels. Of particular interest is the glucose-dependent dual localization of TbPEX13.1, which is involved in the post-translational import of proteins into glycosomes. As previously demonstrated, TbPEX13.1 localizes to glycosomes. However, biochemical fractionations and indirect immunofluorescence of parasites grown in glucose-deplete media suggest that TbPEX13.1 is localized extra-glycosomally and may be associated with the endoplasmic reticulum. Studies are underway to identify residues that regulate extra-glycosomal localization of TbPEX13.1, determine the functional significance of this dynamic localization, and identify other proteins that exhibit dual targeting. In yeast and mammalian cells, peroxisomes can arise de novo from the ER. Such a process has not been described in kinetoplastid parasites and many of the proteins that regulate this process in other systems have not been identified in trypanosome genomes. The presence of a glycosome protein in the ER suggests that this de novo process may occur in kinetoplastid parasites, thereby opening new avenues for drug development.

Gene Family Copy Number Variation Throughout the Cryptosporidia

Rodrigo P. Baptista¹ and Jessica C. Kissinger¹,²,³
¹Center for Tropical and Emerging Global Diseases, ²Department of Genetics and ³Institute of Bioinformatics, University of Georgia, Athens, GA, USA

Cryptosporidium, the causative agent of cryptosporidiosis, is a protozoan parasite with a broad host range including animals and humans. Its public health importance lies in its ability to generate large waterborne outbreaks. It is the second most important pathogen after rotavirus with respect to moderate to severe diarrhea in children. It is also a major veterinary pathogen with a significant impact on the economy. Currently, there are no fully effective drugs or vaccines to treat or prevent cryptosporidiosis. The lack of continuous in vitro culture systems, costly animal models and difficult in vivo genetics make genomics an attractive approach. However, one of the road blocks for treatment development is the lack of reference genome sequences with good experimental data to support the assembly and annotation. Existing genome sequences were generated using short read sequencing platforms resulting in assemblies containing numerous short contigs of unknown order and orientation. It is well known that one of the biggest challenges of short read assemblies is resolving repetitive regions, such as multi-copy gene families, many of which are important for the parasite’s virulence. Repetitive sequence are often compressed or even lost, during the genome assembly process. The main aim of this project was to identify repetitive genes in different Cryptosporidium species using several bioinformatics tools and compare their copy number variation among species and within strains. We were able to detect new multi-copy genes, many of which were thought to be single copy, such as the tryptophan synthase B gene that may play a role in parasite immune evasion. Copy number variation data will help to further our insight into parasite survival mechanisms.
Phosphate Transport by the Acidocalcisome Transporter TbPho91 of Trypanosoma brucei

Evgeniy Potapenko, Ciro Cordeiro, Guozhong Huang and Roberto Docampo

Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602

Acidocalcisomes are acidic calcium stores rich in orthophosphate (P$_i$), pyrophosphate (PP$_i$) and polyphosphate (polyP). We have identified a phosphate transporter (TbPho91) localized to the acidocalcisomes of Trypanosoma brucei, which belongs to the group of parasites that cause African trypanosomiasis. TbPho91 has 12 transmembrane domains, an N-terminal regulatory SPX domain and an anion permease domain and is an ortholog to Saccharomyces cerevisiae vacuolar phosphate transporter ScPho91, and to T. cruzi contractile vacuole phosphate transporter TcPho91. Functional expression in Xenopus laevis oocytes followed by two-electrode voltage clamp showed that TbPho91 is a low affinity transporter with a K$_m$ for P$_i$ in the millimolar range. Application of P$_i$, PP$_i$ or polyP resulted in sodium-dependent depolarization of the oocyte membrane potential and similar results were observed when the yeast and T. cruzi orthologs were expressed. Sodium-dependency was evaluated by replacement with the non-permeable cation N-methyl-D-glucamine. Anion selectivity was demonstrated by applying voltage pulses in the presence of phosphate, sulfate or nitrate. Xenopus oocytes were shown to have robust Na$^+$-dependent $^{32}$P$_i$ uptake. Our results indicate that TbPho91 is a P$_i$ sodium symporter involved in acidocalcisome phosphate homeostasis.

Targeting the NAD Salvage Pathway for the Treatment of Schistosomiasis

Michael D. Schultz¹, Davide Botta¹, Tulin Dadali¹, Anna Manouvakhova³, Melinda I. Sosa³, Sara N. McKellip³, LaKeisha Woods³, Nichole A. Tower³, Larry J. Ross³, Lynn Rasmussen³, E. Lucile White³, Indira Padmalayam³, Wei Zhang³, Corinne Augelli-Szafran³, Maaike Everts⁴, Leonardo Sorci⁵, James R. Bostwick³, Mark J. Suto³ and Frances E. Lund¹

¹Dept. of Microbiology, UAB, Birmingham, AL, USA ²Dept. of Biomedical Engineering, UAB, Birmingham, AL, USA ³Southern Research, Birmingham, AL, USA ⁴Dept. of Pediatrics, UAB, Birmingham, AL, USA ⁵Dept. of Medicine and Surgery, Università Politecnica Delle Marche, Ancona, Italy

The two major pathways of NAD synthesis are the de novo synthesis from amino acid precursors and the salvage pathway in which nicotinamide-containing precursors are recycled into NAD. A comparative genome analysis of S. mansoni, the parasite that causes Schistosomiasis, enabled the assembly of a putative NAD biosynthetic pathway and identified only orthologues of NAD salvage-specific genes. The expression of these genes was subsequently confirmed by PCR. This suggests that NAD biosynthesis in S. mansoni is dependent on salvaging of NAD precursors and not on de novo synthesis. Given the critical role of NAD in cell survival, we hypothesized that inhibition of NAD biosynthesis through the salvage pathway in adult S. mansoni parasites would result in diminution of NAD pools and subsequent toxicity. Indeed, our data show that inhibition of Schistosoma mansoni NAD catabolizing enzyme (SmNACE), a key enzyme in the salvage pathway located on the outer tegument of the parasite, decreased intracellular NAD levels and reduced metabolic activity. Furthermore, pharmacological inhibition of SmNACE significantly reduced the mobility of S. mansoni and induced degeneracy in male parasites, indicative of increased toxicity. A target-based screening campaign led to the identification of several small-molecule inhibitors of SmNACE, and the biological activity on S. mansoni is currently being assessed. Collectively, our data suggest that blockade of NAD biosynthesis is detrimental to S. mansoni, and that targeting the SmNACE-mediated NAD salvage pathway is a promising therapeutic approach for the treatment of Schistosomiasis.
Extracellular Vesicles from *Trypanosoma brucei* Mediate Virulence Factor Transfer and Cause Host Anemia

Anthony J. Szempruch¹, Steven E. Sykes¹, Rudo Kieft¹, Lauren Denison¹, Allison C. Becker¹, Anzio Gartrell¹, William J. Martin², Ernesto S. Nakayasu³, Igor C. Almeida⁴, John M. Harrington⁴ and Stephen L. Hajduk⁴

¹Department of Biochemistry and Molecular Biology, ²Animal Health Research Center, University of Georgia, Athens, GA 30602, ³Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352, ⁴Border Biomedical Research Center, Department of Biological Sciences, University of Texas, El Paso, TX 79968

Intercellular communication between parasites and with host cells provides mechanisms for parasite development, immune evasion and disease pathology. Bloodstream African trypanosomes produce membranous nanotubes that originate from the flagellar membrane and disassociate into free extracellular vesicles (EVs). Trypanosome EVs contain several flagellar proteins that contribute to virulence and *Trypanosoma brucei rhodesiense* EVs contain the serum resistance-associated protein (SRA) necessary for human infectivity. *T. b. rhodesiense* EVs transfer SRA to non-human infectious trypanosomes allowing evasion of human innate immunity. Trypanosome EVs can also fuse with mammalian erythrocytes resulting in rapid erythrocyte clearance and anemia. These data indicate that trypanosome EVs are organelles mediating non-hereditary virulence factor transfer and causing host erythrocyte remodeling inducing anemia.

Dissecting the Movement and Transport Roles of Apicomplexan Dyneins

Ousman Mahmud¹,², Jessica C. Kissinger¹,²,³

¹Department of Genetics, ²Center for Tropical and Emerging Global Diseases, and ³Institute of Bioinformatics, University of Georgia, Athens, GA, 30602

Dyneins are microtubule motor complexes that mediate force and movement. Identification of gene gain and loss patterns by an orthology clustering approach led to the discovery of copy number variation in apicomplexan dyneins. We have performed a phylum-wide characterization of copy number patterns and phylogenetic relationships of the dynein heavy chain (DHC) gene family to identify trends in the evolution of this gene family. Phylogenetic analyses revealed that the last free-living ancestor of the apicomplexans had at least ten different DHC genes. Coccidians have retained all ten ancestral DHC genes. Cryptosporidians, the Piroplasmida and *Gregarinia niphandrododes* each have only one DHC gene, and they are not related to the same ancestral gene. The Piroplasmida DHC gene clusters with the DHCs of red algae and diplomonad excavates. This suggests the Piroplasmida DHC gene may have been acquired via a gene transfer event or they have a copy that has been lost in all other examined apicomplexan species. The differences in the types of DHC genes within the Apicomplexa may reflect differences in microtubule associated transport and movement functions among the parasite lineages. The single-copy DHC genes, especially the one found in the Piroplasmida may have novel or expanded roles. We would like to know how apicomplexans are using their dynein genes especially. Experiments to localize the protein products of DHC genes in *Babesia bovis* and *Toxoplasma gondii* are ongoing.
Modification of a gRNA Design Webtool for CRISPR/Cas9-Based Whole Genome Screens in *Trypanosoma cruzi* and Other Eukaryotic Pathogens

Duo Peng and Rick L. Tarleton
University of Georgia, Athens, GA

The advent of CRISPR/Cas9 genome editing technology has opened the possibility of large-scale forward genetic screens in RNAi-absent organisms such as *Trypanosoma cruzi*. Here, we demonstrate the ability to express gRNAs in *T. cruzi* from a U6-promoter site and to efficiently knockin new gRNAs-encoding oligonucleotides into the gRNA-expression site using site-specific gRNAs and repair templates encoding the gRNA sequence. This technique will enable the creation of a library of gene knock-out *T. cruzi* using a collection of oligo DNA donors encoding target sequence for every gene in *T. cruzi* genome. To assist the design process of such oligo DNA donor libraries, we created a new gRNA batch design mode webtool based on our EuPaGDT gRNA design webtool (available at http://grna.ctegd.uga.edu/batch.html). This batch mode gRNA design webtool can process a list of genes covering whole genomes. For each gene input, the webtool ranks all its gRNAs based on number of genomic on-target and off-target hits and provides a predicted efficacy score. The webtool returns a list of gRNAs consisting of user-specified number of top-ranking gRNA for each input sequence. Users can specify up- or downstream regions of input sequence to design gRNA, permitting disruption of genes closer to transcription start sites. The webtool also produces a list of archetype DNA oligo template for genome editing needs such as batch gene tagging. The batch mode gRNA design webtool can process gRNA design requests for most eukaryotic pathogens and for custom uploaded genomes.

*Trypanosoma cruzi* Specific CD8+ T Cells Continue to Mediate Parasite Control Despite Chronic Antigen Exposure

Angela D. Pack1,3 and Rick L. Tarleton2,3
1Department of Microbiology, 2Department of Cellular Biology, 3Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, United States

Pathogen persistence is often associated with T cell dysfunction as a consequence of continuous antigenic stimulation. *Trypanosoma cruzi* infection is characterized by chronic parasitism of non-lymphoid peripheral tissues and is rarely eliminated despite robust adaptive immune responses. One possible cause of this failure to cure is the relative loss of anti-*T. cruzi* T cell responses over time. In this study, we have evaluated the capacity of CD8+ T cells to mediate control during chronic infection with a focus on T cells at sites of pathogen persistence. Consistent with recent and repetitive antigen exposure during chronic infection, parasite specific CD8+ T cells expressed high levels of KLRG1 with a preferential accumulation of CD69+ cells in skeletal muscle, a niche for *T. cruzi* persistence. A significant proportion of CD8+ T cells in the muscle also spontaneously produced IFNγ, TNFα, and granzyme B in situ, an indication of their detection of and response to *T. cruzi* in vivo. CD8 cell responsiveness to ex vivo stimulation modestly declined over time with the most significant decreases occurring in the TNFα positive population from the muscle. Nevertheless, CD8+ T cells continued to play a crucial role in the management of parasite burden during chronic infection, as exacerbation of parasite load was observed upon depletion of this population. Inhibitory receptor expression has been shown to support pathogen persistence through regulation of T cell function. Attempts to improve T cell function through PD-1 blockade failed to increase effector molecule production or enhance *T. cruzi* clearance. These results highlight the capacity of CD8+ T cells to retain essential in vivo function despite chronic antigen stimulation and support a model in which CD8+ T cell dysfunction plays a relatively minor role in the ability of *Trypanosoma cruzi* to persist in mammals.
Protozoan Hexokinases as Therapeutic Targets: *Trypanosoma brucei* and *Plasmodium falciparum* in the Crosshairs

Stephen L. Patrick¹, Jennifer E. Golden², Varun Dwivedi³, Mark E. Drew³, Elizabeth R. Sharlow⁴, Mindy I. Davis⁵, Min Shen⁵, Matthew D. Hall⁵, Matthew Boxer⁵, Walker M. Blanding¹, Elizabeth W. Kahney¹, and James C. Morris¹

¹Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University, Clemson, SC, ²School of Pharmacy, Pharmaceutical Sciences Division, University of Wisconsin-Madison, WI, ³Departments of Microbial Infection and Immunity and Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH, ⁴Department of Pharmacology, University of Virginia, Charlottesville, VA, ⁵NIH, National Center for Advancing Translational Sciences (NCATS)

Glucose metabolism is critical for the African trypanosome *Trypanosoma brucei* and the malaria parasite *Plasmodium falciparum*. Using heterologous expression of recombinant parasite hexokinases (HKs), we have screened small molecule collections to identify inhibitors, yielding compounds with promising activity against the enzymes and parasites. Through an optimized HTS campaign, a benzamidobenzoic acid inhibitor of *T. brucei* hexokinase 1 (TbHK1) was identified. Development of this scaffold yielded a probe (ML205) that had encouraging potency (< 1uM) against TbHK1 enzyme activity, but lacked efficacy against the bloodstream form trypanosome. Structural modifications of ML205 have improved in vivo activity, yielding potential leads for future development. Interestingly, the benzamidobenzoic acid series lacked activity against the *P. falciparum* HK (PfHK). In the search for inhibitors of the malaria enzyme, ~57,000 compounds were screened (Z'-factor = 0.75 ± 0.08), yielding several structurally related inhibitors. These have been subject to secondary assays against intra-erythrocytic *P. falciparum* parasites, including those that are resistant to chloroquine, and parasites that overexpress PfHK. Additionally, synergy with lopinavir, a *P. falciparum* hexose transporter inhibitor, has been explored. Taken together, these results suggest that protozoan HK inhibitors hold promise in the pursuit of new antiparasitic leads. Research funded by NIH 5 R03 HD081723

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EuPathDB: a Powerful Eukaryotic Pathogen Data Mining Resource with a Unique Search Strategy System

Susanne Warrenfeltz¹ and Jessica Kissinger² for the EuPathDB Team.

CTEGD, University of Georgia, Athens Georgia 30602

The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource supporting over 170 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. EuPathDB facilitates the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed omics data with advanced search capabilities, data visualization and analysis tools.

EuPathDB is a comprehensive resource for mining a wide range of data including genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics and population re-sequencing. Data are analyzed using standard workflows and an in-house analysis pipeline generates data including domain predictions and orthology profiles across all genomes. Our unique strategies system has over 100 structured searches that query the pre-computed data, returning lists of genomic features (e.g. genes, SNPs) that share a biological characteristic (e.g. genes with signal peptides or genes with transmembrane domains). Individual search results can be combined into strategies that refine the biological characteristics of the result (e.g. genes with signal peptides but not transmembrane domains). Nesting and colocation tools provide options for strategy ‘branching’ or interrogating relative genomic colocation (e.g. genes with SNPs in upstream regions). Furthermore, gene results can be transformed into orthologs to make inferences across taxa and extend data mining to organisms with little functional data. This system easily merges evidence from diverse data types and across organisms to place the power of bioinformatics with the entire scientific community.

EuPathDB’s active user support offers an email help desk, social media, a You Tube channel and a worldwide program of workshops. A strategies training module is offered in conjunction with this symposium. To participate, download and complete the tutorial posted at https://goo.gl/NziXmX addressing questions via email to help@eupathdb.org or in person during the symposium poster session. Mark your calendars for a 2-day EuPathDB workshop offered at UGA for local scientists on August 8-9, 2016.
Detection of *Plasmodium* Parasites with Loop Mediated Isothermal Amplification (LAMP) Using Simple Sample Preparation Methods

Reddy V Ponaka, Clarissa Curioso, Naomi W Lucchi, Dragan Ljolje, Denise Patel, Slava Elagin, John W Barnwell, Vladimir Slepnev, Venkatachalam Udhayakumar
Meridian Bioscience, Inc.; Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention

Malaria elimination requires highly sensitive diagnostic tests to enable the accurate and sensitive detection of infected persons including asymptomatic cases. Nucleic amplification methods such as PCR are very sensitive, but are technically challenging to implement in many malaria endemic countries. The loop mediated isotherm amplification (LAMP) method, is well suited for field use as it does not require thermal cyclers to amplify DNA and can be performed using simple and portable equipment. To further facilitate the use of LAMP assays in remote setting, simpler sample preparation methods and lyophilized reagents are required. Here we report on the performance of a simplified malaria assay in an easy to use LAMP platform using pre-dispensed, ambient temperature stable, lyophilized reagents. The illumigene® Malaria LAMP assay, is a *Plasmodium* specific assay and was evaluated using two simple but different sample preparation workflows: the simple filtration (S-PREP) and the column filtration (Malaria PLUS). Laboratory and clinical samples were tested using these workflows to determine the performance of the illumigene® Malaria LAMP. No cross reactivity was observed with the 10 non-*Plasmodium* organisms and human genome tested and the assay was capable of detecting all the five human infecting *Plasmodium* species. Using *P. falciparum* DNA, the limit of detection of the illumigene® Malaria S-PREP and the illumigene® Malaria PLUS method was determined to be 1.95 parasites/µl and 0.44 parasites/µl. Both the Malaria PLUS and Malaria S-PREP generated a sensitivity and specificity of 99% and 100%. The illumigene® Malaria LAMP assay described here has great potential to extend the reach of molecular tools to settings where they are needed without the need for sophisticated sample preparation and with high sensitivity and specificity.

The Origin and Transmission Pattern of Type II Dominant Lineage of *Toxoplasma gondii*

Pooja Saraf and Chunlei Su
Department of Microbiology, College of Arts and Sciences, University of Tennessee

*Toxoplasma gondii* is the most successful zoonotic pathogen known today. PCR-RFLP genotyping analysis of *T. gondii* isolates showed that a few lineages predominate in Europe, Africa, Asia and North America, however, a highly diverse population and lack of dominant lineages were observed in Central and South America. The Type II has been identified as the dominant lineage in Europe, North Africa and North America, but has a low frequency in South America. It is not clear what factors lead to such difference in distribution of the Type II lineage in different geographical region. We hypothesize that the Type II lineage originated in Europe and then transmitted to the New World only recently. To test this hypothesis, we will use 15 Microsatellite markers to analyze Type II strains collected worldwide to determine their within lineage diversity. Accomplishment of this study will allow us better understand transmission patterns and the role of human impact on the expansion of *T. gondii*. 
A Survey of Mississippi Mosquitoes’ Blood Meal and Avian Malaria Parasites

Jessica Aycock, Diana Outlaw
Mississippi State University

Avian malaria, along with many other pathogens, is transmitted through the salivary glands of various mosquitoes. Research on these dangerous vectors has been sorely lacking in the past couple of decades. With increased urbanization of forests, blood meal research has become quite informative. As humans destroy and populate preoccupied environments, various food sources for mosquitoes are no longer available. This potentially results in mosquitoes biting new species of both birds and mammals. This survey will determine the blood meal of various mosquito species throughout counties in Mississippi and identify any malaria parasites carried by the vector. Of the ~27,000 mosquitoes collected, ~200 specimens obtained a viable blood meal for detection. Each mosquito will be identified and processed for DNA extraction. Once the DNA is extracted, three polymerase chain reactions will be performed on the sample. The first will use the mitochondrial cyt b gene to determine the organism from which the blood meal was taken. Each sample will be sequenced to species. The second assay will also use the mitochondrial cyt b gene to detect any malarial parasites. These parasites will also be sequenced and identified to species. The last polymerase chain reaction will be used on select Culex spp. to confirm their identity. The species included are *Culex salinarius*, *Culex erraticus*, *Culex restuans*, and *Culex pipiens* complex.

There are Complex Interactions Between Parasitic Helminths, Drug Treatments and the Host Innate Immune System

Ruby Coates1,2,3, Ciaran J. McCoy1,2, Mary J. Maclean1,2, Barbara J. Reaves1,2, Adrian J. Wolstenholme1,2
1Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA; 2Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602, USA; 3University of Bath, United Kingdom

Anthelminthic Mass Drug Administrations (MDAs) and heartworm prophylaxis rely heavily on diethylcarbamazine (DEC) and macrocyclic lactones (MLs) such as ivermectin (IVM). However, there are large gaps in our understanding about the mode of action of these drugs. There is a clear discrepancy between the mean peak plasma concentrations (Cmax) of IVM and DEC that result in clearance of *Brugia malayi* and *Dirofilaria immitis* microfilariae (Mf) in vivo, and the concentrations required to immobilize them in vitro. For example, the concentration of IVM required to immobilize *D. immitis* Mf in vitro (IC50 of 43 µM) is 14700x higher than the mean peak plasma concentration (Cmax of 3ng/ml) that results in Mf clearance in vivo. These discrepancies are attributed to the involvement of the host immune response in clearance of the Mf. We previously found that peripheral blood mononuclear cells (PBMC) and polymorphonuclear cells (PMN) attach to *D. immitis* Mf in vitro, and increased attachment was observed as IVM concentration was increased. We have also observed that PBMCs and PMNs collaborate to kill *B. malayi* Mf, and attachment of PMNs to *B. malayi* Mf via Neutrophil Extracellular Traps (NETs) has been confirmed. We aim to determine if PBMCs and PMNs can attach to and kill *D. immitis* Mf in vitro, and if their collaboration can result in increased killing. Furthermore, RNAseq of human PBMCs and PMNs treated with IVM and DEC is being performed in order to for us understand the interaction between these drugs and the immune system. The data described here support the hypothesis that there are complex interactions between parasitic helminths, drug treatments and the host innate immune system, which warrant further investigation.
Towards the Development of Next Generation of Rapid Diagnostic Test: Synthesis of Glycophosphatidylinositol (GPI) Analogues of *Plasmodium falciparum* and Immunological Characterization

Bharat P. Gurale¹, Yun He², Xikai Cui¹, Hieu Dinh¹, Abasaheb N. Dhawane¹, Naomi W. Lucchi³, Udhayakumar, Venkatachalam¹,* and Suri S. Iyer *

¹788 Petit Science Center, Department of Chemistry, Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30302. Email: siyer@gsu.edu. Fax: 404-413-5505, ²Atlanta Research and Education Foundation, Atlanta, GA 30329, ³Malaria Branch, Division of parasitic Diseases and Malaria, Center for Global Health, Centers of Disease Control and Prevention, Atlanta, GA 30329

A large number of proteins in malaria parasites are anchored using glycophosphatidylinositols (GPIs) with lipid tails. These GPIs are structurally distinct from human GPIs. *Plasmodium falciparum* GPIs have been considered as vaccine candidates as these molecules are involved in inducing inflammatory responses in human hosts and natural anti-GPI antibody responses have been shown to be associated with protection against severe diseases. The GPIs can also be considered as targets for rapid diagnostic tests. As isolation of native GPIs in large quantities is challenging, development of synthetic GPI molecules can facilitate further exploration of GPI molecules for diagnosis and vaccine development. Here we report synthesis and immunological characterization of a panel of GPI analogues. Three GPI analogues were chemically synthesized and conjugated to a carrier protein to immunize and generate antibodies in rabbits. The rabbit immune sera showed reactivity with synthetic GPIs and native GPIs extracted from *P. falciparum* parasite as determined by Luminex and ELISA based antibody detection methods.

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In vivo Effects of Drugs Used in Lymphatic Filariasis MDA Programs on *Brugia malayi* in Gerbils

Mary J. Maclean¹,², Ashley M. Rogers¹,², Sally M. Williamson¹,², Michael T. Dzimianski¹, Bob S. Storey¹, Adrian J. Wolstenholme¹,²

¹Department of Infectious Diseases, University of Georgia, Athens, GA, United States, ²Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, United States

Lymphatic filariasis (LF) threatens nearly 20% of the world’s population and has handicapped one-third of the 120 million people currently infected. Current control and eventual elimination of LF rely on mass drug administration (MDA) programs with three drugs: ivermectin (IVM), albendazole (ALB), and diethylcarbamazine (DEC). Only the mechanism of action of albendazole is well-understood. For *Brugia malayi*, the in vitro IC₅₀ for ivermectin against microfilariae (Mf) was 6.1 ± 1.1 µM, 120 times the drug concentration that clears Mf from human patients. IC₅₀ values could not be calculated for DEC as no dose response could be observed. These findings suggest that the rapid clearance of Mf observed after treatment with IVM or DEC is aided by the host immune system and does not simply result from the paralysis of the parasites. To gain a better insight into antifilarial drug action, we treated gerbils with patent *B. malayi* infections with 6 mg/kg DEC, 1 mg/kg ALB, or 0.15 mg/kg IVM to mirror the doses used in human MDA programs. These treatments had no effect on the numbers of worms present in the peritoneal cavity of infected animals. Adults and Mf were collected 1 and 7 days post-treatment and RNA was isolated for transcriptomic analysis. The experiment was repeated three times. Preliminary data analyzing the effects of IVM, ALB, and DEC on adults revealed changes in transcripts related to collagen expression, reproduction, embryogenesis, larval development, and lifespan determination. Forthcoming analysis of the effects of these drugs in vivo will provide a better understanding of how they clear filarial parasites.
**From Parasitic Protozoa to Stem Cells and Beyond: The Implications of a Trypanosome Glucosyltransferase in the Field of Mammalian Epigenetics**

Whitney Bullard¹, Rudo Kieft¹, and Robert Sabatini³
³CTEGD, University of Georgia

Hydroxymethyluracil (hmU) is an oxidized DNA base found in the genomes of many organisms. In mammalian genomes, hmU was originally considered to be DNA damage produced from the oxidation of thymine by radical oxygen species (ROS), however, recent work has suggested that hmU can also be generated through enzymatic oxidation of thymine by the ten-eleven translocation (TET) enzymes in mouse embryonic stem cells (mESCs). The function of this enzymatically generated hmU and its location throughout the mESC genome is unknown. Studying this TET generated hmU in mESCs is impeded by the fact that there are currently no methods developed to map hmU within a genome. Here we present a technique, which can be used to specifically enrich for hmU containing DNA sequences using the base J associated glucosyltransferase (JGT) from *Trypanosoma brucei*. JGT is a glucosyltransferase, which catalyzes the conversion of hmU to base J within the Trypanosomatid genome. We demonstrate here JGT can specifically convert hmU to base J in an in vitro reaction. The base J generated from this reaction can then be enriched using an anti-base J antibody pull-down. This technique could be used to map hmU within genomes and may help to shed light on the function of TET-generated hmU within mESCs.

**Investigating the Role of the Notch Signaling Pathway in the Development of Thymic Epithelial Cells**

Cybelle G. Tabilas¹, Julie Gordon¹, Nancy R. Manley³
³Genetics, University of Georgia

The Notch signaling pathway is highly conserved in metazoa and is essential for the proper development of many organs in vertebrates. However, the role of the Notch signaling pathway in the development of the thymus, specifically thymic epithelial cells (TECs), remains unknown. Preliminary data generated by the Manley lab through knocking Notch1 out in the thymus at different developmental stages suggests that Notch1 may be necessary for the maintenance and/or proliferation of thymic epithelial progenitor cells (TEPCs). These data were generated by crossing a Foxn1Cre mouse with a Notch1fx/fx mouse. Foxn1 is TEC specific, therefore using this mouse model results in Notch1 being deleted from all TEC specific cells. To determine whether all Notch signaling was ablated in the Foxn1Cre;Notch1fx/fx mutant thymus, we crossed the mice to a CBF:H2B-Venus reporter mouse. In these mice, Venus, a type of YFP, reports all active Notch signaling in an individual cell. If Notch1 is responsible for all Notch signaling in the thymus, we expect there to be no co-expression of Foxn1 and Venus, therefore no active Notch signaling in any TECs. However, through immunohistochemistry, we saw fewer cells that co-expressed both Venus and Foxn1 in the mutant thymus as compared to the control thymus. These data suggest a variety of things; including a Notch receptor, other than Notch1, has a role in TEC development or cells that co-express both Venus and Foxn1 may be cells that are transitioning from TEPCs to TECs. Future investigation will be required to distinguish between the these or other possibilities.
Identification of New Family of Inorganic Polyphosphate Polyphosphatases in *Trypanosoma brucei*

Ciro Cordeiro, Kyle Ahmed, Brian O. Windle, and Roberto Docampo
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA

*Trypanosoma brucei* belongs to the group of parasites that cause African trypanosomiasis or sleeping sickness. We are interested in understanding the metabolism of polyphosphate in trypanosomes. Previous work in our laboratory reported the presence in these parasites of polyphosphate (polyP)-rich acidocalcisomes and identified a polyP kinase (TbVtc4) involved in the synthesis and translocation of this polymer to acidocalcisomes. There are two identified enzymes involved on the catabolism of polyP in *T. brucei*: TbPPX1 and TbVSP1. Here we report a new family of polyphosphatases in *T. brucei* called Nudix hydrolases. Nudix proteins are versatile enzymes that participate in various pathways and they can hydrolyze phosphoanhydride bonds from various substrates. Interestingly, a Nudix hydrolase from yeast possess polyphosphatase activity. Here we report the identification and characterization of Nudix polyphosphatases as exopolyphosphatases. There are five orthologs in *T. brucei*. These enzymes degrade long chain polyP to shorter length polyP and completely hydrolyze polyP into pyrophosphate and inorganic phosphate. NH2 (Tb427.05.4350) has been found in the glycosome proteome and has a peroxisomal (glycosomal) targeting signal (PTS): SSL, and maximal activity at acidic pH. We characterized the kinetic constants of NH2 in polyP hydrolysis. We also determined that *T. brucei* Nudix hydrolases do not participate in metabolism of inositol pyrophosphates, in contrast with the yeast Nudix hydrolase Ddp1.

*Plasmodium falciparum* ER Resident Protein, PfGRP170, is Required for Asexual Growth and Gametocyte Formation

Heather M. Bishop1,2 and Vasant Muralidharan1,2
1Dept. of Cellular Biology, 2Center for Tropical and Emerging Global Diseases, University of Georgia

In order to make a suitable habitat for growth, the malaria parasite must drastically remodel the permeability, rigidity, metabolism, and membrane features of the parasitized red blood cell. Furthermore, the parasite must create and maintain membrane bound structures such as the parasitophorous vacuole, digestive vacuole, and apicoplast. To accomplish these processes the parasite synthesizes over 1,000 proteins in the parasite cytoplasm and transports them across multiple cellular compartments. The mechanisms that are responsible for export between these topological phases are poorly understood. In *Plasmodium*, all trafficked proteins, except those bound for the mitochondria, begin their journey in the ER. How these proteins are sorted in the parasite’s ER is a vital, unanswered question in the biology of the malaria parasite that has clinical significance. In other eukaryotic organisms protein folding and trafficking is a process driven by molecular chaperones. In addition to protein trafficking, ER chaperones have been shown to play a major in stress response pathways. Interestingly, *Plasmodium* parasites possess minimal ERAD and UPR pathways, despite being subjected to high fevers by the host. Therefore, we are interested in uncovering the roles of ER chaperones in protein trafficking and managing cellular stress. We used a degradation domain based functional knockdown approach to interrogate the function of an ER-resident putative chaperone, PfGRP170. Knockdowns were unable to grow and were less tolerant to brief heat shock stress. Our results also suggest that PfGRP170 is required for gametocyte formation and therefore, essential for both asexual growth and parasite transmission.
Enhancing Host Immune Response to *Trypanosoma cruzi* Through Expression of PAMPs and DAMPs

Phil Y. Yao¹, Samarchith P. Kurup², and Rick L. Tarleton¹
¹CTEGD, University of Georgia, ²University of Iowa

Upon infection of mammalian hosts, *Trypanosoma cruzi* elicits a relatively weak innate immune response and a consequently slow adaptive immune response. We have hypothesized that these characteristics are a result of the relative absence of exposed pathogen associated molecular patterns (PAMPs) on invading *T. cruzi* and the failure to generate host damage associated molecular patterns (DAMPs) during the initial host cell invasion process. In support of this hypothesis we have shown that the continuous expression of *Salmonella* flagellin (FliC), a potent PAMP, results in enhanced *T. cruzi*-specific adaptive immune responses and improved pathogen control. To further explore the role of DAMPs and PAMPs in the anti-*T. cruzi* immune response, we investigated the impact of expression of the host high mobility group box 1 (HMGB1) on *T. cruzi* infection. HMGB1 was selected because it is a protein DAMP and has been shown to activate a number of host pattern recognition receptors involved in innate immunity. *T. cruzi* expressing HMGB1 were produced and HMGB1 expression confirmed by immunofluorescence microscopy. The biological activity of *T. cruzi*-produced HMGB1 was confirmed by the ability of these transgenic parasites to strongly activate caspase-1 in macrophages in vitro. In order to investigate whether co-expression by *T. cruzi* of FliC and HMGB1 will additively or synergistically boost the innate immune responses, we are exploring several methods of producing multiple foreign proteins in *T. cruzi*, including adapting a multicistronic expression system using the 2A peptide. Additionally, modifications of HMGB1 to alter its localization and oxidation state are being used to optimize HMGB1 activation of innate immune response. The impact of these modifications on the adaptive immune response to *T. cruzi* and parasite control in vivo will be explored.

**Wolbachia Protein Wbm0076 Modulates Yeast Actin Dynamics**

Michael K. Mills, Vincent J. Starai
Department of Microbiology, University of Georgia

*Brugia malayi* are nematodes that can live in the human lymphatic system for 6-8 years, causing the chronic disease state filariasis. It has been shown that *B. malayi* can only cause filariasis when it harbors the intracellular bacterial endosymbiont, *Wolbachia pipientis*; clearance of the bacterium from its host effectively eliminates the pathogenicity of the nematode and therefore understanding the mechanisms by which *W. pipientis* survives within *B. malayi* will provide important information regarding both new drug targets for treatment of filariasis, and interactions between symbiotic bacteria and their hosts. *W. pipientis* produces a number of secreted proteins (termed WSPs) that are thought to direct its survival within the nematode host, but characterizing these WSPs in the lab has proven difficult due to the fact that *W. pipientis* cannot be grown in pure culture. Therefore a surrogate eukaryotic model system, the budding yeast *Saccharomyces cerevisiae*, can be used to identify WSPs which alter conserved eukaryotic pathways. Bioinformatics suggests that the Wbm0076 protein belongs to the eukaryotic WAS family of proteins involved in regulating actin dynamics via the activation of Arp2/3. Expression of Wbm0076 in yeast strains results in an increase of cortical actin patch formation as measured by fluorescently-tagged Abp1p or Abp140p proteins localized to cortical actin patches and actin cables respectively. Furthermore, we find that very high level expression of Wbm0076 is lethal to wild type yeast, but yeast strains lacking Abp1p, an Arp2/3-binding protein, are resistant to Wbm0076 toxicity. Taken together, these data suggest that Wbm0076 modifies Arp2/3 activity and actin dynamics in yeast and activity is likely conserved during survival within *B. malayi*. Future experiments will uncover the mechanisms by which *abp1* strains are resistant to Wbm0076 activity, and whether Wbm0076 directly modulates actin polymerization in vivo and in vitro.
Development of a Mouse Model for Pregnancy Maintenance During Maternal Malaria Infection

Catherine Smith¹ and Julie Moore¹
¹CTEGD, Infectious Diseases, University of Georgia

Placental malaria, a severe form of complicated malaria that impacts pregnant women, causes chronic maternal illness, pregnancy loss, low birth weight, and is responsible for as many as 300,000 infant deaths a year in Sub-Saharan Africa. The exact mechanisms underlying the placental damage and poor birth outcomes observed in placental malaria are incompletely understood. Due to the unavailability of human placental tissue prior to term, mouse models that recapitulate the features of placental malaria are required to further our understanding of placental malaria pathogenesis. Mouse models utilizing Plasmodium chabaudi and Plasmodium berghei both result in pregnancy loss when infection is initiated in early pregnancy. Therefore, we intend to establish a mouse model for pregnancy maintenance following infection in early pregnancy.

Investigation of the Chaperone Activity of Polyphosphate in Trypanosomes

Raquel S. Negreiros¹, Noelia Lander², Guozhong Huang³, and Roberto Docampo⁴
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602

Inorganic polyphosphate (polyP) is a polymer of three to thousands of high-energy phospho-anhydride-bonded phosphate units, and is universally conserved. Trypanosomes contain acidocalcisomes which are membrane-bounded organelles packaged with large quantities of polyP complexed with Ca²⁺, Mg²⁺, Zn²⁺, basic amino acids, and polyamines. Several studies have shown that bacteria or unicellular eukaryotes lacking polyP are more sensitive to different stress conditions, including heat shock, osmotic stress, starvation, and reactive oxygen species. Recently, polyP was identified in bacteria as a global, highly effective chaperone that stabilizes proteins, prevents protein aggregation both in vitro and in vivo, and maintains proteins in a refolding-competent form. In this context, we investigated whether polyP acts as chemical chaperone in Trypanosoma brucei. PolyP was able to protect T. brucei proteins from thermal aggregation in a dose-dependent manner, suggesting it also acts as chemical chaperone in eukaryotic cells. The thermal aggregation of the model enzymes luciferase, citrate synthase and malate dehydrogenase was also inhibited in the presence of polyP. The incubation of T. brucei and T. cruzi lysates with biotinylated polyP results in the binding of glycosomal enzymes to this polymer. To investigate the role of glycosomal polyP as chemical chaperone in vivo, we designed a vector to deliver an exopolypophosphatase to the glycosomes of procyclic forms. The exogenous exopolypophosphatase was active and localized in the glycosomes of transfected parasites. Both wild type and transfected parasites showed similar levels of short chain polyP, possibly due to the low level of polyP in the glycosomes compared to that in the acidocalcisomes. To evaluate the levels of inactivation in different cellular compartments after heat shock, luciferase was targeted to the cytosol or the glycosomes of T. brucei procyclic forms and their localization was confirmed.
Spermatogonial stem cells (SSCs) are crucial for life-long fertility. These cells are present in the testes along with many other somatic cell types. Proposed treatments would require SSCs to be cultured in vitro for a period of time as well as cryopreserved. Optimal conditions for culturing of human SSCs have yet to be determined. Often cultures are quickly overrun with contaminating somatic cells. Removal of these somatic cells and the enrichment of spermatogonia is important step in culturing of the cells. One method of removing contaminating somatic cells is through the use of magnetic activated cell sorting (MACS). Using this method we were able to enrich for spermatogonia in culture. The effectiveness of our freezing procedure and the sorting was evaluated using previously validated germ cell specific markers FGFR3, UTF1, and PLZF. Freezing and thawing of tissue decreases the number of viable cells, but does not affect overall germ cell viability. Although germ cells are surviving proportionally to somatic cells, our method of cryopreservation needs to be optimized to increase cell yields. Cryoprotectants, such as DMSO and sucrose are necessary for the survival of cells when freezing and thawing. Our current methods of freezing/thawing are not compatible with the use of sucrose as a cryoprotectant.

Trypanosoma brucei is the eukaryotic pathogen responsible for African Sleeping Sickness in humans and Nangana in cattle. The dense surface protein coat of T. brucei allows the parasite to adapt to host immune system defenses. Fatty acids comprise the membrane-anchoring domain of these surface coat proteins and thus are a key resource. T. brucei can synthesize fatty acids de novo, but they also take them from their host, though little is known about the latter process in T. brucei. We hypothesize that fatty acyl-CoA synthetases (ACSs) mediate fatty acid uptake, as they trap free fatty acids intracellularly by converting them to fatty acyl-CoAs, which cannot cross membranes. T. brucei possesses 5 ACSs: ACS1-4 and a long-chain ACS, LACS5. The ACS genes are highly similar and previously published work showed they have overlapping specificities. Thus, we generated a procyclic RNA interference cell line designed for tetracycline-inducible knock-down all 5 ACSs (panACS RNAi). While panACS RNAi induction led to a noticeable growth defect, we observed no effect on uptake of fluorescently-labeled fatty acids (BODIPY-C12 and BODIPY-C16). It is possible that ACSs are not important in fatty acid uptake. Alternatively, the panACS RNAi was not efficiently knocking down the ACS(s) governing fatty acid uptake. To determine the efficiency of RNAi knock-down for each ACS gene, we have optimized RT-qPCR conditions for all 5 ACS genes and are currently quantifying the degree of knock-down after induction of panACS RNAi. These studies will allow us to determine which, if any, ACS genes play a role in fatty acid uptake in T. brucei. Our long-term goal is to define the molecular mechanisms of fatty acid uptake to improve our understanding of the relationship between trypanosomes and their hosts.
Base J Represses Genes at the End of Polycistronic Gene Clusters in *Leishmania major* by Promoting RNAP II Termination

David L. Reynolds¹, Brigitte T. Hofmeister², Laura Cliffe¹, T. Nicolai Siegel³, Britta A. Anderson⁴, Stephen M. Beverley⁴, Robert J. Schmitz⁵, and Robert Sabatini¹

¹Department of Biochemistry and Molecular Biology, University of Georgia, ²Institute of Bioinformatics, University of Georgia, ³Research Center for Infectious Diseases, University of Wuerzburg, ⁴Department of Molecular Microbiology, Washington University School of Medicine, ⁵Department of Genetics, University of Georgia

The genomes of kinetoplastids are arranged into polycistronic gene clusters that contain multiple functionally unrelated genes that are co-transcribed from a single initiation site. Because of this genome arrangement, it is thought that all gene expression regulation occurs at post-transcriptional levels. Recently however, multiple epigenetic modifications have been found enriched at transcription initiation and termination sites in kinetoplastids, including histone variants and the modified DNA base J (beta-D-glucosyl-hydroxymethyluracil) that suggests mechanisms of transcriptional control. We have shown previously that in *L. major* the loss of J leads to a defect in RNA Polymerase (RNAP) II termination resulting in transcription into opposing (convergent) gene clusters and production of antisense RNAs. It has remained unclear whether such termination defects negatively impact gene expression and whether they are linked to the essential nature of base J in *Leishmania* spp. In contrast to the expectations often attributed to antisense transcription, we find that J reduction and subsequent read through (antisense) transcription into adjacent gene clusters does not significantly negatively impact sense mRNA abundance. Instead, J reduction results in upregulation of mRNAs from genes at the end of specific gene clusters. More than half of the upregulated genes are located downstream of or within a genomic region enriched with base J, suggesting that read through transcription occurs upon the loss of base J within gene clusters. We refer to this process as the promotion of gene cluster internal termination by base J, that is, termination occurs prior to the end of a gene cluster thereby repressing expression of the downstream gene(s). These findings suggest that the essential nature of J in *Leishmania* spp. is due to its role in repressing genes specifically at the end of gene clusters, rather than preventing transcriptional interference arising from read through and dual strand transcription at convergent gene clusters.

Cholesterol Localization and Ecdysteroidogenesis in the Mosquito *Aedes aegypti*

David McKinney, Michael Strand, Mark Brown
Department of Entomology, University of Georgia

The yellow fever mosquito, *Aedes aegypti*, is the vector for the arboviruses that responsible for yellow fever, chikungunya, and dengue fever. The acquisition of a blood meal is necessary for both the vectoral mechanism and mosquito reproduction. Following a blood meal the mosquito ovaries are activated by neuropeptides to produce the steroid hormone ecdysone, which is contingent on cholesterol as a precursor. Previous work has shown that insects lack the ability to produce cholesterol de novo, and that there are sufficient sterol reserves in the ovaries for ecdysone production following activation in vitro. Using the sterol stain filipin, and the sterol manipulator methyl-β-cyclodextrin we examined sterol location and movement in the mosquito ovary with respect to blood feeding and ecdysteroidogenesis. Here we demonstrate that the steroidogenic tissues are the principle site of free sterol storage in the female mosquito, and within the ovaries the follicle cells and nurse cells possess most of the sterol specific signal. Additionally, TopFluor cholesterol was used to assess sterol allocation by the mosquito. TopFluor showed rapid reallocation in the mosquito. Sterol storage and regulation in mosquitoes is dynamic, and the distribution of free sterols was heavily weighted towards the steroidogenic tissues.
Prior Exposure Seasonal H1N1 Influenza Shapes Specificity of Pandemic H1N1 Influenza Antibody Responses and Virus Evolution

Nicole S. Williams¹, Donald Carter Jr.¹, Ted Ross¹
¹Department of Infectious Diseases, University of Georgia

**Background:** Human antibody responses against the 2009 pandemic H1N1 (pH1N1) influenza virus are predominantly directed against conserved epitopes in the stalk and receptor-binding domain of the hemagglutinin (HA) protein. This is in stark contrast to pH1N1 antibody responses generated in ferrets, which are focused on the variable Sa antigenic site of HA. Here we hypothesize that humans have born in different era elicit antibodies directed at different epitopes on the pH1N1 HA head after infection. Humans born in different eras are exposed to different seasonal H1N1 (sH1N1) that have varying homology to pH1N1 in the HA antigenic sites. Upon infection of pH1N1 the antibody responses can be shifted to different epitopes in the HA head which is dependent on an individual’s exposure to sH1N1 that have homology in the same antigenic sites.

**Methodology/Principal Findings:** To test this hypothesis ferrets were infected either with a single sH1N1 representing different antigenic eras followed by pH1N1, or sequentially infected with sH1N1’s and then challenged with pH1N1 influenza. It is shown here ferrets infected with single sH1N1 strains from eras between 1934 and 2007 do not elicit cross-reactive neutralizing titers to pH1N1 influenza prior to challenge, whereas ferrets sequentially infected will produce broadly neutralizing antibody titers that will neutralize the pandemic strain. Serum collected after pH1N1 challenge was analyzed against pH1N1 virus containing mutations in homologous residues found in sH1N1 strains to determine the antibody specificity after challenge with pH1N1. We observed differences in the pre-exposed ferrets depending on prior sH1N1 exposure.

**Conclusion/Significance:** An individual’s antibody repertoire after exposure to pH1N1 influenza virus is influenced by prior sH1N1 exposure. Conserved residues in seasonal and pandemic strains direct the antibody response to different epitopes depending on an individual’s era of exposure.

A Unique Insight into the MiRNA Profile During Genital Chlamydial Infection

Tankya Simoneaux¹, Yusuf Omosun¹, Yuehao Wu¹, Khamia Ryans¹, Danielle McKeithen¹, Debra Ellerson, Roshan Pais¹, Francis O. Eko¹, Carolyn M. Black², Jessica Kissinger, Uriel Blas-Machado, Joseph U. Igietseme¹,², and, Qing He¹
¹Department of Microbiology, Biochemistry & Immunology, Morehouse School of Medicine, Atlanta, and ²Centers for Disease Control & Prevention (CDC) Atlanta GA USA.

**Background and Significance:** *Chlamydia* pathogenesis leads to changes in gene expression, which causes deleterious changes within the environment of the female genital tract. These changes include pelvic inflammatory diseases, salpingitis and tubal factor infertility. All of these conditions can obstruct the female genital tract causing infertility in women. **Objectives:** To identify novel and differentially expressed miRNA in the genital tract of chlamydia infected mice that are associated with chlamydial pathogenesis. **Methods:** Mice were divided into two groups: First, primary infection (one infection) and second, secondary infection (two infections). Mice were sacrificed and genital tract tissues were collected at four time points; one, two, four, and eight weeks after infection. miRNA sequencing, pathology, cytokine analysis and fertility assay were performed. **Results:** Secondary infected mice had higher bacteria loads and their infection lasted longer than primary infected mice. Pathological presentation showed that secondary infected mice had more ectasia and inflammation in the oviduct and uterus. miRNAs were differentially expressed in both primary and secondary infection, however the miRNA profile was different for both groups. Pathway analysis showed that the differentially regulated miRNA might be regulating endothelial migration and focal adhesion of cells, extracellular matrix receptors, and adherens junction formation amongst other functions. In addition, we also discovered novel miRNAs that were only found in chlamydia-infected mice. **Conclusion:** The progression of the chlamydial pathogenesis depends on host factors, which include miRNAs; we intend to determine to what extent these miRNAs could be regulating the outcomes that were observed in *Chlamydia* infection.
Investigating Early Infection Status of the Filarial Parasite *Brugia malayi* in the Cat, the Laboratory Model for Lymphatic Filariasis

Erica J. Burkman, Katherine E. Hogan, Molly D. Savadelis, and Andrew R. Moorhead  
Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Human lymphatic filariasis (LF) is a mosquito-borne disease primarily caused by the parasitic nematodes *Wuchereria bancrofti* and *Brugia malayi*. These parasites are a major cause of morbidity globally, with an estimated 120 million people infected. *Brugia malayi* is the preferred laboratory model for LF due to *W. bancrofti* requiring the use of primate hosts. Currently, the domestic cat is utilized as the primary non-rodent animal model for *B. malayi*. However, on average only 25%-50% of felines become patent, so a method of early detection would be invaluable. Currently, the only test to determine infection status is the detection of circulating microfilariae, which are usually detectable 4–6 months post-infection. In other filarial parasites such as *Dirofilaria immitis*, the Enzyme Linked Immunosorbent Assay (ELISA) is used to detect circulating female uterine antigen. Recently, it was suggested that heat treatment of serum or plasma may dissociate the antibody-antigen complex, potentially releasing the antigen so that it may be detected. Due to the close relationship of these filarial worms, there could be detectable cross-reactivity after heat treatment for *B. malayi* antigen in these capture-antibody tests. We hypothesized that we would be able to detect circulating antigen after heat treatment in the serum of these infected cats. Ten male domestic cats were infected by subcutaneous injection of 400 *B. malayi* third-stage larvae. Serum was collected at key time points post-infection. Both heat-treated and room temperature serum was tested for circulating antigen using the DiroCHEK® ELISA kit. Of the six cats that became microfilaremic, five tested antigen-positive, whereas only one cat with a low microfilaremia tested antigen-negative. These data may indicate a methodology other than microfilarial counts may be used to detect *B. malayi* infections in cats. Furthermore, heat treatment of serum could expose epitopes that cross-react with the antibody used in commercial *D. immitis* tests.

A Central Role for the Ubiquitin-Like Protein Apiquitin in Apicoplast Protein Import in *T. gondii*

Justin Fellows; Michael Cipriano; Swati Agrawal; Boris Striepen  
Department of Cellular Biology, University of Georgia, Athens, GA 30602

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that is the causative agent of the prevalent disease toxoplasmosis. *T. gondii* has a unique organelle called the apicoplast which is derived from a red algal endosymbiont. It is essential for the parasite as the compartment for fatty acid, heme and isoprenoid biosynthesis. The majority of the approximate 500 apicoplast proteins are nuclear encoded and have to be imported across the four membranes that surround the apicoplast. Import across the second outermost membrane (periplastid membrane) of the apicoplast depends on an apicoplast specific endoplasmic reticulum associated protein degradation (ERAD) complex and enzymes of the associated ubiquitination cascade. However, ubiquitin associated with these apicoplast specific enzymes has long been elusive. Here we identify apiquitin an apicoplast protein that has significantly altered from its ubiquitin ancestor. We demonstrate that apiquitin combined with the AAA ATPase CDC48AP act as the recognition complex that pulls apicoplast proteins through the periplastid membrane during protein import. We constructed conditional null mutants and use genetic complementation to show that both proteins are essential for parasite survival and are critical for import across the periplastid membrane of the apicoplast. Apiquitin is distinct from other known ubiquitin-like proteins and bioinformatic analysis suggests a clade specific to Apicomplexans. Our experiments support a model under which apiquitin is transiently transferred to imported apicoplast proteins to act as the signal for the AAA ATPase to drive import across the periplastid membrane.
Generation of a Recombinant NKp46 Protein to Evaluate Influenza Hemagglutinin (HA) Protein Binding to NK Cell Receptors

Ivelisse Resto-Garay, David L. Rose, S. Mark Tompkins and Kimberly D. Klonowski*
Department of Infectious Diseases and *Cellular Biology, University of Georgia, Athens, GA

NKp30, NKp44, and NKp46 are part of a family of NK cell receptors that directly recognize diverse pathogens including Mycobacterium and Plasmodium spp, and viruses including herpes, pox, New Castle, and influenza. However, the contribution of NK cell antigen-specificity to health in the steady-state and their overall therapeutic and vaccine potential have been largely ignored. This is mainly due to significant knowledge gaps regarding how NK cells participate and regulate immune responses through these pathogen-specific receptors. Our project focuses on the NK cell activating receptor NKp46, which specifically binds influenza hemagglutinin (HA) protein. Distinct influenza subtypes and strains have been shown to differentially activate NK cells via NKp46 ligation, however the features of the HA that influence NKp46 binding are unknown. The goal of our research is to identify the determinants of influenza HA binding to NKp46 and subsequent activation, with the long-term goal of understanding how differential activation via NKp46 signaling impacts both immediate NK cell activation and subsequent anti-viral immune responses. We hypothesize ligand specificity associated with human and avian influenza HAs results in differential binding to NKp46 and subsequent NK cell activation. To test this, we generated a fusion protein construct with the extracellular domain of human NKp46 linked to the human IgG1 Fc (NKp46-Ig). Similar constructs have been shown to bind NKp46 ligands and inhibit NK cell activation. The construct was then stably transfected into HEK 293 cells and a Sandwich ELISA confirmed its expression in the culture supernatants. Currently, we are using the recombinant NKp46-Ig to assess binding of this receptor to a library of human (H1 & H3) and avian (H5 & H7) HAs with defined receptor specificities. Subsequently, we will use this protein to validate the specificity of NKp46 in regulating human NK cell activation by HA-expressing and influenza-infected cells in vitro.

Elevated Dopamine Levels and Reduced Dopamine Turnover in the Striatum After Toxoplasma gondii Infection

Jeffrey B. Eells¹, Shirley Guo-Ross¹, Derek Bramlett¹, David S. Lindsay² and Andrea Varela-Stokes¹
¹Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, ²Department of Biomedical Science & Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

Infection with the protozoan parasite Toxoplasma gondii has been linked with an increased risk of several neuropsychiatric disorders, including schizophrenia, obsessive compulsive disorder and attempted suicide. Additionally, T. gondii infection is associated with more severe symptoms and exacerbated pathological changes in the brains of schizophrenia patients. In rodents, T. gondii infection alters behaviors that include elevated activity, reduced levels of anxiety and loss of aversion to cat urine. One mechanisms proposed through which T. gondii alters behavior is via effects on dopamine neurotransmission. Previous studies have shown that T. gondii can increase whole brain dopamine levels and that two tyrosine hydroxylase enzymes, the rate limiting step in the synthesis of dopamine, are expressed by T. gondii. The current study was designed to investigate the relationship between striatal dopamine levels and localization of T. gondii cysts. Mice were infected with T. gondii and euthanized for 8 weeks later. The striatum was isolated from one side of the brain for the measurement of dopamine and metabolites. Tissue sections through the striatum from the other side of the brain were stained with H&E and examined for T. gondii cysts. Toxoplasma gondii infected mice had significantly higher tissue dopamine levels but reduced dopamine turnover (DOPAC/Dopamine) as compared to uninfected controls. On tissue sections, T. gondii cysts were identified in the cortex, but no cysts were observed in the striatum of infected mice. These data demonstrate that T. gondii infection can alter dopamine neurotransmission. Toxoplasma gondii cysts, however, are unlikely to be the direct source of the elevated dopamine levels as a substantial number of cysts would be needed to significantly add to the high dopamine production in the striatum. Neuroinflammation or direct effects on dopamine neurons are other potential mechanisms through which T. gondii infection could alter dopamine neurotransmission.
Differential Sensitivity to Benznidazole among *Trypanosoma cruzi* Isolates is Associated with Isolate-Specific Tissue Tropism

Fernando Sánchez-Valdéz, Angel Marcelo Padilla, Juan Bustamante & Rick Tarleton
CTEGD, University of Georgia, Athens, Georgia, USA

Although benznidazol (BNZ) is the most widely used drug for treating *Trypanosoma cruzi* infection, variable efficacy and frequent side effects limits its utility. The high failure rate of BNZ is due in part to the natural resistance of some *T. cruzi* isolates. However, in vitro assays show these so-called BNZ-“resistant strains” have similar sensitivity to BNZ as in vivo “susceptible” strains, suggesting that factors other than the intrinsic drug resistance mechanism may influence the persistence of these strains after standard BNZ treatment. Here we test the hypothesis that a differential tropism of “resistant strains” for specific tissues could be a determinant factor in the BNZ resistance of certain isolates. In agreement with in vitro data, we show that luciferase-expressing parasites of “resistant” or “susceptible” lines are cleared at similar rates by BNZ in a focalized subcutaneous infection model. However, when tested in a short treatment model of systemic infection, “resistant” strains show a slower rate of clearance and persisted in tissues in which “susceptible” strains were not detectable, even by quantitative PCR. Additionally, after a full standard-dose BNZ treatment, parasites of “resistant” strains were detected in various locations, including the gut, adipose tissue and heart, while the detection of parasite DNA of “susceptible” strains was restricted to the gut. These results support the hypothesis that a differential tropism or tissue-specific persistence contributes to BNZ resistance in vivo. Additionally, these studies suggest that isolates traditionally considered “susceptible” might also persist at very low levels in the gut following BZN treatment.

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Characterization of Mode of Action of Hoechst Analogs on Bloodstream Form *T. brucei*

1Serena Walker, 2Nihar Ranjan, 1Brady Russo, 1Sunayan Ray, 2Dev Arya, and 1Kimberly Paul
1Dept. of Genetics & Biochemistry, Clemson University, Clemson, SC 2Dept. of Chemistry, Clemson University, Clemson, SC

*Trypanosoma brucei* is an Eukaryotic parasite that causes Human African Trypanosomiasis, or African Sleeping Sickness, in humans and nagana in animals. *T. brucei* is a member of the class kinetoplastida and have a single large mitochondrion, a kinetoplast, with a unique DNA structure that may be a prime candidate for drug targeting. To look into this possibility, a series of analogs of the DNA minor-groove intercalating dye, Hoechst, were synthesized. The EC50s of each Hoechst analogs against bloodstream form wild-type (BF WT) 427 *T. brucei* were determined, with EC50s ranging in the micromolar to low nanomolar range. The 4 most potent compounds were examined further for their effect on *T. brucei* cell cycle. BF WT 427 cells were cultured in the presence of each compound at the EC50 concentration. The cells were then DAPI stained and analyzed through fluorescence microscopy to see how the drug may be affecting cell division. Approximately 100 cells were counted in each of three trials for both cells grown in the presence of the compound and cells grown with DMSO as a solvent control. One of the compounds (DPA 151) appeared to disrupt the replication of the kinetoplast DNA (kDNA), with a large proportion of dyskinetoplastlastic cells, which are cells with nuclear DNA but no kDNA. Due to the unique structure of the kDNA, if the effect of the drug is selective to the kDNA over the nuclear DNA, it may also be selective for *T. brucei* cells over host cells.
Understanding the Roles of Chloroquine Resistance Transporter Homolog (TgCRT) in the Pathogenesis of Toxoplasmosis

Paige Teehan, Zhicheng Dou
Department of Biological Sciences, Clemson University, Clemson, SC

Chloroquine resistance transporter (CRT) mutations have been shown to facilitate the expulsion of chloroquine from *Plasmodium falciparum* digestive vacuoles. This resistance has made chloroquine ineffective as an antimalarial drug. *Toxoplasma gondii* expresses an ortholog of CRT, named TgCRT, associated with a lysosome-like digestive organelle known as the vacuolar compartment or plant-like vacuole (VAC/PLV), indicating that the VAC/PLV is an equivalent digestive vacuole structure in *Toxoplasma* parasites. TgCRT-null mutants displayed the VAC/PLV swollen approximately 10-fold larger than that in wild type parasites. In addition, the ablation of the TgCRT gene resulted in a reduction of parasite replication, attenuation of parasite virulence, and more strikingly, a 10-fold reduction of neural tissue cysts during chronic infection. This study plans to assess the physiological alterations within the VAC/PLV in TgCRT-null parasites, such as changes in pH or homeostasis of metal ions. Furthermore, we plan to assess the roles of the VAC/PLV in the pathogenesis of toxoplasmosis, such as utilization of macromolecular nutrients and resistance to osmotic pressure change in the environment.

Two Mechanisms of Ca\textsuperscript{2+} Entry in *Toxoplasma gondii*

Miryam A. Hortua Triana, Karla Marques Nogueras and Silvia N.J. Moreno
Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA

*Toxoplasma gondii* is an obligate intracellular parasite that infects and replicates in an extensive variety of cell types. During its lytic cycle, *T. gondii* actively invades host cells, creating a parasitophorous vacuole, where it divides to finally exit in search of a new host cell. Parasite invasion is a highly coordinated and active process involving several discrete steps. Considering the enormous Ca\textsuperscript{2+} concentration gradient (~20,000-fold) to which tachyzoites are exposed upon egress (100 nM in the host cytosol vs. ~1.8 mM in the extracellular space), it seems a feasible hypothesis that the extracellular stage represents an opportunity to use extracellular Ca\textsuperscript{2+} as a mechanism to enhance Ca\textsuperscript{2+}-dependent invasion processes as well as to replenish intracellular Ca\textsuperscript{2+} stores. We propose that there are two mechanisms of calcium entry in *T. gondii*: one that is voltage-gated and sensitive to nifedipine, a voltage gated calcium channel blocker, which responds rapidly to changes in ionic environment. A second mechanism would become activated by a signaling cascade involving calcium, a phospholipase C and a protein kinase G (PKG). We believe that a TRP-like channel could be responsible for this second permeation pathway. We also propose that both mechanisms interact with each other. In an attempt to characterize these two mechanisms, we used a number of pharmacological tools like PIPLC, PKG and TRP channel inhibitors. We also tested a phosphodiesterase inhibitor, zaprinast that leads to activation of PKG because of the resulting increase in its activator, cyclic GMP. We used Fura-2AM loaded parasites to measure cytosolic calcium concentrations as well as GCAMP6 expressing parasites. In addition, molecular tools like PIPLC, TRP channel knockout mutants as well as PKG mutants insensitive to the inhibitor compound 2 were used.
Elucidating the Role of Trehalose-6-Phosphate Synthase in the Development and Transmission of Cryptosporidium parvum

Emily K. Myers¹, Mattie C. Pawlowic¹, Sumiti Vinayak¹, Carrie F. Brooks¹, Boris Striepen¹,²
¹Center for Tropical and Emerging Global Diseases, University of Georgia, 30602 ²Department of Cellular Biology, University of Georgia, 30602

Cryptosporidium is an apicomplexan parasite that infects the gastrointestinal tract of many animals. Infection with Cryptosporidium parvum or Cryptosporidium hominis is the second leading cause of severe diarrhea in young children worldwide. No vaccine exists for cryptosporidiosis, and the only approved drug does not benefit those most in need of treatment—immunocompromised individuals and young children. The largest impediments to studying Cryptosporidium are lack of continuous in vitro culture system and the lack of a system for genetic modification. Our lab recently developed tools to genetically modify the parasite using CRISPR/Cas9 and developed a mouse model of infection. For this project, we examined the role of trehalose-6-phosphate synthase (t6ps) in oocyst formation, which is the hardy, transmitted life cycle stage of Cryptosporidium. The oocyst protects the parasites from environmental stresses during transmission and is a complex structure composed of lipids, sugars, and proteins. The T6PS enzyme catalyzes the last two steps of trehalose synthesis. Trehalose is a dimer of glucose made by most organisms, excluding mammals. Trehalose protects against desiccation in other organisms, is known to be a virulence factor in Mycobacterium, and is a known component of the Cryptosporidium oocyst. Using our CRISPR/Cas9 and mouse infection model, we attempted to directly knockout t6ps in the parasite. We were unable to obtain transgenics, suggesting that this gene is essential for parasite survival. Then, we endogenously epitope tagged the gene and confirmed cytosolic expression of t6ps, and that the gene is more highly expressed in females. Finally, we verified the biochemical functionality of the t6ps enzyme through a complementation test in Saccharomyces cerevisiae and Escherichia coli mutants lacking the orthologous gene.

Identification and Characterization of a Pex3-Like Protein (TbP3L) in T. brucei

Logan Crowe and Meredith Teilhet-Morris
EPIC, Clemson University

Trypanosoma brucei is a protozoan parasite that causes human African trypanosomiasis. Current drugs are toxic and difficult to administer necessitating the search for new drug targets. Glycosomes are essential, peroxisome-like organelles that compartmentalize a many metabolic pathways. Like peroxisomes, glycosome homeostasis is maintained through the action of proteins called peroxins (Pexs). Glycosome homeostasis can be divided into four processes: organelle biogenesis via ER, post-translational protein import, organelle multiplication, and degradation via pexophagy. One protein, Pex3, is essential to peroxisome membrane protein import as well as ER-dependent peroxisome maturation, and is present in all eukaryotes that harbor peroxisomes. To date however, a Pex3 homolog has not been identified in trypanosomes. Pex3 directs protein import through interactions with Pex19 via a 15 amino acid sequence that is conserved in yeast, mammals and plants. Using this sequence to query the T. brucei genome database, we identified a Pex3-like protein that we have named TbP3L. We have expressed a fluorescently tagged copy of this gene; however, expression of this fluorescently tagged protein appears to be controlled by an unknown mechanism. Silencing of TbP3L via RNAi alters expression of a glycosome marker protein, and as of yet, we have not been able to knock out P3L. Our findings suggest that TbP3L may be a Pex3 homolog. If true, TbP3L may be a marker for studying glycosome biogenesis from the ER. While this pathway has been hypothesized to occur in T. brucei, it has not been directly demonstrated.
**Lipid Droplets in the Trypanosomatid *Crithidia fasciculata***

Myles Chetcuti and Kimberly Paul  
E.P.I.C., Clemson University, S.C.

Lipid droplets are dynamic organelles central to lipid metabolism. Lipid droplets are composed of a core of cholesterol and neutral lipids surrounded by a phospholipid monolayer. Embedded in the lipid droplet membrane are proteins that regulate lipid droplet dynamics. Little is known about lipid droplets in trypanosomatids. Examination of the trypanosomatid genomes in TriTrypDB revealed no homologs to known lipid droplet structural proteins, such as caveolin or perilipin. To date, only one lipid droplet protein is known in *Trypanosoma brucei*: a Lipid Droplet Kinase that is localized to the lipid droplet surface and regulates lipid droplet number.

Our aim is to use the model trypanosomatid *Crithidia fasciculata*, an obligate parasite of mosquitoes, to characterize lipid droplet number and intracellular distribution during parasite growth in culture. We collected *Crithidia* cells from different growth phases in culture: lag phase, early logarithmic, and late logarithmic growth. We stained the lipid droplets with Nile Red, a fluorescent lipophilic dye, counter-stained the nuclei and kinetoplast DNA with DAPI, and imaged cells using fluorescence microscopy. Lipid droplet numbers per cell were determined by visual inspection of images obtained. Both the number and intracellular distribution of lipid droplets changed over the course of growth in culture. In the early phase of growth in culture, many cells had no or a single lipid droplet, while cells in late log phase exhibited a higher number of lipid droplets. In addition, lipid droplets frequently located at the posterior pole opposite the flagella. Our future work will focus on the biochemical purification of lipid droplets from *Crithidia*, with the goal of identifying the trypanosomatid lipid droplet machinery.

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**Understanding Transport in *Cryptosporidium parvum***

Mattie C Pawlowic¹, Emily K Myers¹, Carrie F Brooks¹, Boris Striepen¹²  
¹CTEGD, University of Georgia, Athens, GA ²Department of Cellular Biology, University of Georgia, Athens, GA

*Cryptosporidium* is a leading cause of diarrheal disease worldwide, especially for young children and immunocompromised adults. Even in less severe cases, chronic infection and malnutrition often lead to stunting and developmental delays. The only drug approved to treat cryptosporidiosis is not effective in children and there is no vaccine. Lack of tools to study this pathogen, including the lack of continuous culture, greatly hinders the understanding of its unique biology and impedes the development of much needed therapies. Recently, we developed a system to genetically modify *C. parvum* that utilizes CRISPR/Cas9 to target specific genes and a murine model to propagate parasite mutants. We are particularly interested in using these tools to better understand the biochemistry of the host-pathogen interaction. *C. parvum* infects the cells that line the small intestine and occupies an intracellular but extracytoplasmic niche. This provides access to both the host and the lumen of the gut. Therefore it is of little surprise that the *C. parvum* genome lacks most synthetic pathways in favor of numerous transporters. At the interface with the host cell, the parasite contains a membranous structure called the “feeder organelle.” We are systematically localizing *Cryptosporidium* transporters to understand 1) how *Cryptosporidium* salvage nutrients from the host and gut, and 2) to characterize the role of transporters in the feeder organelle. We will report the development of tools to tags transporters, the localization of transporters, and progress in developing a conditional expression system. By studying *C. parvum* transporters, we hope to identify essential aspects of the host-pathogen interaction that can be leveraged for therapy and other applications.
SAR-Guided Optimization of Anti-Trypanosomal Aurones

Jeannie Moore Stubblefield, Zachary Evan Taylor, Scott Handy, Anthony L. Newsome
Tennessee Center for Botanical Medicine Research, Middle Tennessee State University, Murfreesboro, TN

Aurones, discovered over seventy years ago, are a class of molecules belonging to the flavonoids family of natural products. Due to the discovery that some aurone derivatives can serve as potent biological agents and the ease through which they can be accessed synthetically, interest in aurone derivatives has increased in recent years. A library of aurone derivatives was synthesized to probe the aurone scaffold against in vitro models for trypanosomal diseases. The library incorporates a wide range of functionality including bioisosteres and exploration of different levels of lipophilicity. Structure activity relationship analysis followed by successive manipulations of the aurone scaffold produced compounds with improved anti-trypanosomal activity (Trypanosoma brucei IC50 <10 µM), low toxicity (selectivity >10), and cross-species activity (Trypanosoma cruzi and Leishmania amazonensis).

Regulation of Base J Synthesis in Kinetoplastids

Rudo Kieft, Melissa Jennings and Robert Sabatini
Department of Biochemistry & Molecular Biology, University of Georgia

Leishmania spp. are protozoan parasites that cause leishmaniasis, which can affect humans in parts of Africa, Asia, South America, and Central America. Leishmania spp. are part of the order Kinetoplastida, which are responsible for various diseases like Human African Trypanosomiasis (T. b. gambiense and T. b. rhodesiense) and Chagas disease (T. cruzi). All kinetoplastids contain a novel modified DNA base, called base J (a glucosylated thymine residue). The localization of base J within various kinetoplastid genomes is restricted to telomeric DNA and, more importantly, regions where RNA Polymerase II initiation and termination occurs. It has been shown that alterations in J levels in these specific regions can lead to differences in gene expression, which may play a role in the pathogenicity of these organisms. However, little is understood how J synthesis is regulated at these specific sites within the genome. The first step in base J synthesis is the hydroxylation of thymine residues by a thymidine hydroxylase (JBP1 and JBP2), followed by glucosylation by a (non-specific) Glucosyl Transferase (GT) of this HmU residue. Understanding the mechanisms which regulate the specificity of J synthesis within the kinetoplastid genome is essential to understand its role in (epi) genetic regulation of gene expression. We hypothesize that additional proteins associated with the JBPs are involved in directing the specificity of the thymine modification within the kinetoplastid genome. We have therefore tagged JBP1 and JBP2 with a dual affinity tag (a streptavidin binding protein domain and a protein A domain) for purification from Leishmania tarantolae extracts to identify possible interacting proteins. This may then lead us to understand the specific nature and function of this modified base within the genomes of these parasites.
Exploring the Role of Small Non-Coding RNA During Zika Infection

Juliana Assis Geraldo¹,², Francislon Silva de Oliveira¹, Victor Satler Pylro¹, Fabiano Sviatopolk-Mirsky Pais¹, Juliane Dutra Medeiros¹, Jack Gilbert³,⁴,⁵, Angela Volpini¹, Gabriel da Rocha Fernandes¹
¹Genomics and Computational Biology Group, René Rachou Research Center (CPqRR-FIOCRUZ), Belo Horizonte, MG, 30190-002, Brazil. ²Center for Tropical and Emerging Global Diseases. ³Department of Ecology and Evolution, The University of Chicago, Chicago, Illinois, USA. ⁴Argonne National Laboratory, Institute for Genomic and Systems Biology, Argonne, Illinois, USA. ⁵Department of Surgery, The University of Chicago, Chicago, Illinois, USA

Zika virus (ZIKV) is an emerging mosquito-borne flavivirus, first isolated in 1947 from the serum of a pyrexial rhesus monkey caged in the Zika Forest (Uganda/Africa). In 2007 ZIKV was reported to be responsible for an outbreak of relatively mild disease, characterized by rash, arthralgia, and conjunctivitis on Yap Island, in the western Pacific Ocean. In the past year, ZIKV has been circulating in the Americas, probably introduced through Easter Island (Chile), by French Polynesians. In early 2015, a new outbreak was recognized in northeast Brazil, where concerns over its possible links with infant microcephaly have been discussed. Providing a definitive link between ZIKV infection and birth defects is still a big challenge. MicroRNAs (miRNAs), are small noncoding RNAs that regulating post-transcriptional gene expression by translational repression, and play important roles in viral pathogenesis and brain development. It is estimated that more than 60% of human protein-coding genes contain at least one conserved miRNA-binding site. The potential for flavivirus-mediated miRNA signaling dysfunction in brain-tissue development provides a compelling mechanism underlying perceived link between ZIKV and microcephaly. Here, we provide strong evidences toward understanding the mechanism in which miRNAs can be linked to the “congenital Zika syndrome” symptoms. We also mapped the small RNA from Aedes aegypti vector infected and no-infected with Zika virus against the ZIKV genome. We found piRNA associated with transposons control and cleaving of mRNA. Moreover, following World Health Organization (WHO) recommendations, we have assembled a database that can be used to help target mechanistic investigations the possible relationship between ZIKV symptoms and miRNA-mediated human gene expression, helping to foster potential targets for therapy.

Comparative Analysis of Illumina and Hybrid Assembly Annotations for Cryptosporidium baileyi

Shelton Griffith¹,², Rodrigo Baptiste², Jessica C. Kissinger¹,²,³
¹Institute of Bioinformatics, ²Center for Tropical and Emerging Global Diseases, and ³Department of Genetics, University of Georgia, Athens, GA, 30602

Diarrhea is one of the leading causes of death among children under five globally. More than one in ten child deaths – about 800,000 each year – is due to diarrhea. Today, only 44% of children with diarrhea in low-income countries receive the recommended treatment, and limited trend data suggest that there has been little progress since 2000. Cryptosporidium is a microscopic parasite that is the second leading cause of early childhood diarrheal illness globally. There is no fully effective drug treatment or vaccine for Cryptosporidium, and the basic research tools and infrastructure needed to discover, evaluate and develop interventions for this parasite are mostly lacking. My project uses comparative genomics to assess the suitability of a related, easy to culture bird-infecting species as a possible model for study. However, before I perform my comparative analysis I must first determine whether an Illumina genome assembly or a Hybrid (PacBio, Illumina) assembly is best. To make this assessment, I have conducted annotations of both assemblies of C. baileyi. Once I determine which genome assembly is the best, my annotation will allow me to compare the C. baileyi genome sequence to other Cryptosporidium and apicomplexan species. I anticipate that my work will reveal information about conserved Cryptosporidium genes as well as species-specific sequence changes that may warrant further investigation.
Assessing the Role of PfERC in the Regulation of Calcium in P. falciparum

Manuel Fierro, Andrea Hortua, Silvia Moreno, Vasant Muralidharan
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA

Plasmodium falciparum is the causative agent of malaria, and it is estimated that more than 200 million people in the world are infected every year with half a million of those cases resulting in death. Calcium (Ca$^{2+}$) is an important secondary messenger cells and has been implicated to be important in the progression of the intraerythrocytic lifecycle of malaria. Eukaryotic cells are able to regulate Ca$^{2+}$ through the means of channels or signaling cascades that release from or replenish Ca$^{2+}$ stores or from the outside environment, but little is known about how Plasmodium regulates Ca$^{2+}$. Genomic analysis shows that Plasmodium lacks many of the channels (voltage-gated Ca$^{2+}$ channels, STIM/ORI complex) or signaling cascade partners (IP$_3$ receptor, ry-anodine receptor) that are common for regulating Ca$^{2+}$ in other organisms. However, there are many calcium binding proteins annotated in the genome, some of which reside in the endoplasmic reticulum (ER). One of these is Endoplasmic Reticulum-resident Calcium Binding Protein (ERC). PfERC has 6 predicted EF-hands and previous studies have shown its localization to the ER. We hypothesize that PfERC is an essential protein needed in buffering the available Ca$^{2+}$ found in the ER and thus serving a purpose in regulating Ca$^{2+}$. In order to determine this, we employed a conditional knock-down system using the ribozyme glmS which is activated upon binding to glucosamine-6-phosphate (GlcN6P). Our results show that ERC appears to be essential since ablation of the protein with this system begins to kill the parasite after 24 hours with no effect to the M9, a mutated version of glmS, control.

Understanding the Development of the Sexual Stages of Cryptosporidium parvum

Jayesh Tandel$^{1,2}$, Adam Sateriale$^1$, Carrie Brooks$^1$ and Boris Striepen$^{1,2}$
$^1$CTEGD, University of Georgia, $^{1,2}$Department of Cellular Biology, University of Georgia

Cryptosporidium parvum is an apicomplexan parasite with a monoxenous life cycle. Sexual reproduction in the parasites results in the transition of macrogametes into oocysts. Oocysts are then capable of infecting the same host or can be transmitted to another host via oral-fecal route. Hence, sexual reproduction is an important pre-requisite to achieve host to host transmission.

In order to develop transmission blocking strategies against C. parvum, it is important to understand the development of the sexual stages of C. parvum. Our strategy involves developing sex-specific reporter strains of C. parvum, and understanding genes that are uniquely expressed in the sexual stages. Our analogous strategy is to delve in to the roles of ApiAP2 transcription factors in sexual stage commitment.
Zinc is an essential element that acts as a cofactor for a large number of enzymes and regulatory proteins. Zinc must be tightly regulated, because both Zn\(^{2+}\) deficiency and high concentrations of intracellular free Zn\(^{2+}\) (nanomolar) are deleterious for cells. All organisms have evolved complex mechanisms to regulate cellular Zn\(^{2+}\). The total concentration of cellular Zn\(^{2+}\) typically ranges from 0.1 to 0.5 mM, however the concentration of free Zn\(^{2+}\) is approximately at picomolar levels, because most Zn\(^{2+}\) is bound to proteins and/or compartmentalized in intracellular compartments. *Toxoplasma gondii* is an obligate intracellular parasite that replicates inside a host-cell during the tachyzoite lytic cycle. How *T. gondii* obtains the Zn\(^{2+}\) needed for intracellular replication while in the low free Zn\(^{2+}\) environment of the host cytosol is unknown. One hypothesis is that tachyzoites uptake Zn\(^{2+}\) prior to invasion, while it is extracellular, where the concentration of free Zn\(^{2+}\) is approximately 1000-fold higher. The tachyzoite would sequester Zn\(^{2+}\) to avoid any toxicity. A vacuolar type zinc transporter (TgZnT, TGME49_251630) and two putative zinc-iron permeases (TgZIP2 and TgZIP4) were C-terminal tagged and localized using immunofluorescence (IFA) and cryoimmuno electron microscopy (TgZnT). We observed labeling of the plant-like vacuole (PLV) in extracellular tachyzoites by all three proteins, and punctate vesicular labeling during intracellular replication. A polyclonal antibody against TgZnT also showed this localization and both ZIPs co-localized with TgZnT to varying degrees. A fluorescent zinc indicator showed Zn\(^{2+}\) labeling of the TgZnT compartment. Deletion of TgZnT in tachyzoites caused reduced parasite growth and exogenous expression of TgZnT in *Saccharomyces cerevisiae* and *Xenopus laevis* oocytes, provides evidence of Zinc transport. These results support our hypothesis that TgZnT is responsible for sequestration of Zn\(^{2+}\) in the PLV after egress, while the TgZip2 and TgZIP4 facilitate the distribution of the stored Zn\(^{2+}\) to necessary compartments during subsequent intracellular replication.

Resolution of Genome Duplications in *Toxoplasma gondii*

Shubham Basu and Jessica C. Kissinger
Center for Tropical and Emerging Global Diseases and Institute of Bioinformatics, University of Georgia

Copy number variations have long been linked to differences in phenotype and virulence of many pathogens. An analysis of available sequence data from 62 strains of *Toxoplasma gondii* has revealed an excess of SNPs and an excess of sequence reads mapping to distinct genome locations when compared to the reference genome sequence from strain ME49. These observations are indicative of a presence of multiple repetitive regions (CNV’s) in the reads being mapped and/or a collapsed repetitive region in the ME49 reference genome sequence. In addition, strain-specific SNP and CNV differences are observed. In contrast, bioinformatics analysis of the currently available genome sequence of *Toxoplasma gondii* ME49 shows no segmental duplications, a highly anomalous result that might be indicative of collapsed and/or merged repetitive regions, a common artifact of genome assembly algorithms. This critical assembly error has greatly hampered the ability of researchers to discover and study SNP and CNV’s associated with virulence. The aim of the current research is to identify and disambiguate these repeats using long-read single molecule sequences that span the genome sufficiently well to cover duplicated regions. The resolution will focus on several key strains of *Toxoplasma gondii* with an emphasis on reference strain ME49. Resolved sequences will then be compared across strains to catalog extent and type of replicated regions and the affected genes. This study will provide a new reference genome sequence with greatly reduced assembly errors and much needed insight into the scope and potential significance of genome duplications and SNPs in the evolution of *Toxoplasma gondii* strains.
**Biomarkers for Schistosomiasis: a Promising Schistosoma mansoni Egg Protein to Effectively Diagnose Acute and Chronic Clinical Forms in Brazil**

Vanessa Silva Moraes¹,², Paulo Marcos Zech Coelho², Donald A. Harn¹, Flavia Fernanda Bubula Couto², Neusa Araujo², Sueleny Silva Ferreira Teixeira², William de Castro Borges³, Rafaela Fortini Queiroz Grenfell¹,², Lisa M. Shollenberger¹

¹Department of Infectious Diseases, University of Georgia; ²Rene Rachou Research Center, Fiocruz, Brazil; ³Federal University of Ouro Preto, Brazil

Schistosomiasis is a serious public health problem. The standard for diagnosis is the Kato-Katz method, which has low sensitivity and does not work well on patients with low-level infections, representing the majority of cases. Adding tests such as ELISAs using soluble egg antigens (SEA), increases diagnostic accuracy in low burden areas of Minas Gerais, Brazil. However, crude SEA antigens have low-specificity and cross-react with other helminthes. Therefore, the goal of this work is to identify SEA proteins with high schistosomiasis specificity, as well as the sensitivity to differentiate between active (acute and chronic) and cured (post-treatment phase) infections, in order to develop point-of-care (POC) tests as well as improve others’ diagnostic methodologies. To complete these studies, SEA was generated from mice 45 days after infection with *Schistosoma mansoni*. Using a protocol approved by the Brazilian Ethical Committee, human serum was obtained in Minas Gerais from each group: healthy volunteers (negative controls); schistosome acute, chronic and post-treatment patients; and patients infected with other helminthes. The sample from each group were submitted to two-dimensional Western blot (2D-WB) using native and sodium metaperiodate (SMP) treated SEA. The immunoreactive spots were identified by mass spectrometry. A total of 23 spots were identified by serum from *Schistosoma* infected patients. Among these, 22 spots were identified by serum from patients infected with other helminths, and 9 by negative control samples. One spot was uniquely recognized by sera from *Schistosoma*-infected patients and detection remained after sugar denaturation by SMP, suggesting serum antibodies were binding to peptide epitopes. We identified a potential egg protein from this unique spot, for which we are developing monoclonal antibodies, toward the development of POC test and highly specific ELISAs. Further, the fast, simple POC assay requires minimal equipment and will be an accurate screening tool for epidemiologic surveying in low resource regions.

**Xfp1 Motif's 473RIFS'P477 Phosphorylation is Crucial for Cryptococcus neoformans Virulence**

Satyanarayana Lagishetty and Kerry Smith
Eukaryotic Pathogens Innovative Center, Department of Genetics and Biochemistry, Clemson University, South Carolina-29634

*Cryptococcus neoformans* is an invasive-opportunistic pathogen of the central-nervous-system and the most frequent cause of fungal meningitis, infecting about one-million people/year and killing ~625,000/year-worldwide. At present general fungicides like flucanazole and amphotericin B for *Cryptococcus* meningitis are given for treatment, which are toxic to human cells. Acetate metabolism is important during infection, but its significance is unknown. We have identified two potential pathways for acetate production and enzymes from these pathways have been shown to be up-regulated during infection. One of two possible pathways is composed of the xylulose-5-phosphate/fructose-6-phosphate phosphoketolase1 (XFP1) - acetate kinase (Ack) pathway. Xfp1 and xfp2 knockout out mutants display reduced survival rate in mouse macrophages. We have previously produced recombinant *C. neoformans* XFP1 (CnXFP1) in *E. coli* using various expression vectors with different purification tags, but the enzyme, although soluble, lacks activity. The cAMP-PKA secretes virulence factors, which recognizes ‘RXXSP’ motif and phosphorylates the Serine residue. The ‘RXXSP’ motif is completely conserved among XFP1 sequences but is universally replaced by Gly residue in bacterial XFP’s. In order to obtain phosphorylated Ser476 of Cn-XFP1, we utilized a system developed by the Rinehart lab for producing site-specific Serine phosphorylated proteins. The recombinant pSer476 Cn-XFP1 was active and our kinetic studies indicate that the enzyme displays substrate cooperatively with fructose-6-phosphate but not with inorganic phosphate. 3D structure prediction of Cn-XFP1 shows the biological functional unit for XFP1 is ~194KD dimer and the active site is at the interface between two monomers. The pSer476 regulates the Cn-XFP1 activity by govern catalytic Glu534 residue. We are obtained XFP1 crystallization conditions hits and rest of X-ray crystallography work is in progress. Further, we also expressed the XFP1 in eukaryotic expression system and our studies conclude that Xfp1’s are specifically phosphorylated by cAMP-PKA, which mainly regulates the virulence factors in *Cryptococcus neoformans*. 
Quantitative in silico Approaches to Inferring Sexual Recombination Events Among *Toxoplasma gondii* Populations

Brent Allman¹², Juan Gutierrez³⁴, and Jessica Kissinger¹³⁵

¹Center for Tropical and Emerging Global Diseases, UGA, ²Post-Baccalaureate Research Education Program, UGA, ³Institute of Bioinformatics, UGA, ⁴Department of Mathematics, UGA, ⁵Department of Genetics, UGA

*Toxoplasma gondii* is a zoonotic apicomplexan parasite with a broad host range among warm-blooded animals, leading to a global distribution. The parasite is the cause of toxoplasmosis, a significant health risk to pregnant women and the immunocompromised. Until recently, *T. gondii* was believed to exhibit an almost exclusively clonal population structure, consisting of few sexual recombination events. However, the *Toxoplasma* research community analyzed the genomes of 62 *T. gondii* strains; their analyses suggest that the population structure involves more sexual recombination than previously thought. Since the parasite can only undergo sexual recombination in the felid gut, it is difficult to know how frequently different strains of *T. gondii* meet to facilitate sexual recombination. A better understanding of sex at the population level can be informative of how rapidly markers of pathogenesis may be moving throughout the population. To do this experimentally or in the field is prohibitively costly and prone to sampling error. In order to quantify these events, we use two approaches, (i) an in silico population simulation, and (ii) a matrix clustering analysis using Matlab. For the in silico population simulation, we generate multiple hypothetical sub-populations in the form of simulated genome sequences using software for forward-genetics simulation. Then at different rates of sexual recombination and inbreeding, we generate new progeny populations. These in silico progeny are then compared to the measured natural *Toxoplasma* population structure. From this, we will infer the rate of sexual recombination occurring in the natural population. The matrix clustering analysis will allow us to distinguish between individuals that are the result of sexual events, and those that are the result of clonal expansion (both sexual [inbreeding], and asexual). This comparison will allow us to construct proposed groups of sexually produced progeny.