1 Exploring the male-induced female reproduction of Schistosoma mansoni in a novel medium Ípíng Wang1, Rui Chen1, James Collins1 1) UT Southwestern Medical Center.

Schistosomiasis is a neglected tropical disease caused by schistosome parasites that infect over 200 million people. The prodigious egg output of these parasites is the sole driver of pathology due to infection. Female schistosomes rely on continuous pairing with male worms to fuel the maturation of their reproductive organs, yet our understanding of their sexual reproduction is limited because egg production is not sustained for more than a few days in vitro. Here, we explore the process of male-stimulated female maturation in our newly developed ABC169 medium and demonstrate that physical contact with a male worm, and not insemination, is sufficient to induce female development and the production of viable parthenogenetic haploid embryos. By performing an RNAi screen for genes whose expression was enriched in the female reproductive organs, we identify a single nuclear hormone receptor that is required for differentiation and maturation of germ line stem cells in female gonad. Furthermore, we screen genes in non-reproductive tissues that maybe involved in mediating cell signaling during the male-female interplay and identify a transcription factor gli1 which knockdown prevents male worms from inducing the female sexual maturation while having no effect on male/female pairing. Using RNA-seq, we characterize the gene expression changes of male worms after gli1 knockdown as well as the female transcriptional changes after pairing with gli1 knockdown male worms. We are currently exploring the downstream genes of this transcription factor that may mediate the male stimulus associated with pairing. Taken together, these data provide the platform to study schistosome sexual development in vitro and develop new strategies to control schistosome egg production.

2 Shifting perspectives: a modification to the life cycle of Trypanosoma brucei Jaime Lisack1, Sarah Schuster1, Ines Subota1, Markus Engstler1 1) Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany.

African trypanosomes are the causative agent of Human African Trypanosomiasis (HAT) and the cattle plague, Nagana. As with all vector-borne diseases, transmission is intimately tied to parasite survival and propagation in the vector, the blood-sucking tsetse fly. Two main stages of T. brucei live in the mammalian host, the proliferative long slender form and the cell cycle arrested short stumpy stage. The transition from slender trypanosomes into stumpy occurs via a quorum sensing mechanism, mediated by the parasite-excreted stumpy induction factor (SIF). As slender populations grow, the SIF threshold is reached and stumpy trypanosomes form. Aside from morphological and metabolic changes, stumpy trypanosomes also express the protein associated with differentiation 1 (PAD1) (Matthews, 2009). The switch from slender to stumpy trypanosomes is thought to accomplish two things. First, it auto-regulates parasite density and hence, prolongs survival of the host. Second, stumpy forms are thought to be ‘pre-adapted’ to survival in the tsetse fly vector. It has long been believed that upon uptake from the mammalian blood, only the ‘pre-adapted’ stumpy trypanosomes can survive in the fly midgut, while slender trypanosomes were thought to die. Keeping slender trypanosome populations below the SIF threshold and diluting parasites at different densities for in vivo fly infections, we show that both slender and stumpy trypanosomes can propagate with comparable rates in the tsetse fly. We amassed a largest dataset of fly infections and dynamics, further showing that that only one trypanosome, slender or stumpy, is necessary to infect a tsetse fly. Next, we looked at differentiation hallmarks at the early stages of differentiation, both in cell culture and in the fly. Here, we found that upon differentiation, PAD1, though to indicate stumpy formation in the mammalian host, is expressed during slender trypanosome differentiation in the fly midgut, without cell cycle arrest or morphological transition to the stumpy stage. Thus, both stumpy and slender cells can complete the life and transmission cycle inside the tsetse fly vector. These results not only hold implications regarding the life cycle of T. brucei but also on transmission dynamics. This data could help answer the long-held question of how disease incidence can be sustained in chronic mammalian infections, at low blood parasitemia, where stumpy trypanosomes are characteristically absent.

3 Critical Role for Isoprenoids in Apicoplast Biogenesis by Malaria Parasites Megan Okada1, Russell Swift2, Kritthika Rajaram2, Hans Liu2, John Maschek1, Sean Prigge2, Paul Sigala1 1) University of Utah School of Medicine; 2) Johns Hopkins School of Public Health.

Isopentenyl pyrophosphate (IPP) is an essential metabolic output of the apicoplast organelle in Plasmodium malaria parasites and is required for prenylation-dependent vesicular trafficking between discrete subcellular compartments. We have elucidated a critical and previously uncharacterized role for IPP in the biogenesis of the apicoplast organelle itself. Inhibiting IPP synthesis blocks apicoplast elongation and inheritance by daughter merozoites. Exogenous IPP and polyrenols rescue these defects. Knockout of the only known isoprenoid-dependent apicoplast pathway, TRNA prenylation by MiaA, does not affect blood-stage parasites and cannot explain apicoplast reliance on IPP. However, we have localized a previously annotated, but uncharacterized, polyprenyl synthase (PPS, PF3D7_0202700) to the apicoplast lumen. PPS knockdown is lethal to parasites, rescued by IPP, and blocks apicoplast biogenesis, and these observations are sufficient to explain the reliance of apicoplast biogenesis on IPP synthesis. PPS was previously proposed to additionally catalyze the initial phytoene synthase (PSY) step in carotenoid synthesis. However, we found no evidence for PSY function by PF3D7_0202700 or de novo carotenoid synthesis by blood-stage Plasmodium. We hypothesize that PPS synthesizes polyrenols critical for membrane expansion during apicoplast biogenesis. This work provides a new paradigm for isoprenoid utilization in malaria parasites and identifies a novel essential branch of apicoplast metabolism suitable for therapeutic targeting.

4 The Cryptosporidium single-cell atlas reveals key life cycle stages and a commitment to male and female development Katelyn Walton1, Jayesh Tandel1, Jodi Gallicksrud1, Stephen Carro1, Eoin Whelan1, Elise Krespan1, Daniel Beiting1, Boris Striepen1 1) University of Pennsylvania, Philadelphia, PA.

The apicomplexan parasite Cryptosporidium parvum is a leading global cause of diarrheal disease and infects millions of people each year, with a particularly high prevalence in south Asia and sub-Saharan Africa. The current treatment, nitazoxanide, is ineffective in immunocompromised patients and malnourished children, and there is no vaccine. Therefore, a great need exists for new and more effective therapeutics against Cryptosporidium. Transmission of the parasite occurs via the fecal-oral route, and the entire life cycle takes place in a single host: asexual growth, replication, and division take place in intestinal epithelial cells, followed by transition to a male or female form and sexual reproduction. Yet while a few molecular markers have been identified to demarcate this life cycle progression, the signaling pathways and the gene expression changes involved in development remain largely unknown. Here, we used single-cell RNA sequencing of infected cultures and mice to determine the complete life cycle transcriptome of Cryptosporidium parvum in vitro and in vivo. Analysis of 9,310 individual parasite transcriptomes revealed clear asexual cycle progression with an abrupt switch to either male or female development during the trophozoite stage. We find no transcriptional evidence for a type II meront, as gene expression dramatically changes only when transition to male or female occurs and none is noted in the prior asexual cycle. In asexual parasites, gene expression was driven by cell cycle progression dominated early by ribosomal biogenesis, processing, and assembly followed by protein folding and then DNA replication. Later asexual stages expressed many secreted proteins, including an abrupt transition to invasion related organelles. While females arrest in vitro, in vivo they progress to sporoconversion, and sporozoite and merozoite gene expression is highly similar. Importantly, single-cell transcriptional profiling revealed stage-specific and sex-specific expression of AP2 and Mby transcription factors, including distinct expression in early males, outlining a pathway for sex-specific commitment. Future work will focus on determining the functional roles of these transcriptional regulators. Overall, our work provides the first comprehensive view of Cryptosporidium gene expression over the entire life cycle and identifies the gene regulatory networks that control them.

5 N6-methyladenosine in poly(A) tails stabilize VSG transcripts Idálio Viegos1, Juan Macedo1, Mariana De Niz1, João Rodrigues2, Francisco Aresta-Branco3, Samir R. Jaffrey4, Luisa M. Figureiredo1 1) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa; 2) Clarify Analytical, Rua das Mercadores 128A, 7000-872 Évora, Portugal; 3) Division of Immune Diversity, German Cancer Research Center, Im Neuenheimer Feld 280, 69120, Heidelberg, Germany; 4) Department of Pharmacology, Weill Medical College, Cornell University, New York, NY, USA.
RNA modifications are important regulators of gene expression. In Trypanosoma brucei, transcription is polycistrionic and thus most regulation happens post-translationally. N6-methyladenosine (m6A) has been detected in this parasite, but its function remains unknown. Here we show that ~50% of the m6A is located in the poly(A) tail of the monoallelically expressed Variant Surface Glycoprotein (VSG) transcript. m6A residues are removed from the VSG poly(A) tail prior to deadenylation and mRNA degradation. Using genetic tools, we identified a 16-mer motif in the 3′ UTR of VSG that acts as a cis-acting motif for inclusion of m6A in the poly(A) tail. Removal of this motif from the VSG 3′ UTR results in poly(A) tails lacking m6A, rapid deadenylation and mRNA degradation. To our knowledge this is the first identification of an RNA modification in the poly(A) tail of any eukaryote, uncovering a novel post-transcriptional mechanism of gene regulation.

6 Identification of an m6A-binding YTH protein regulating protein synthesis in the human malaria parasite

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Post-transcriptional regulation of gene expression is central to the development and replication of the malaria parasite, Plasmodium falciparum, within its human host. The timely coordination of RNA maturation and protein synthesis relies on the recruitment of specific RNA-binding proteins to their cognate target mRNAs. One possible mediator of such mRNA-protein interactions is the N6-methylation of adenosines (m6A), a widespread mRNA modification of parasite mRNA transcripts. Here, we used RNA protein pull-downs, RNA modification mass-spectrometry and quantitative proteomics to identify two YTH-domain proteins (PLYTH.1 and PLYTH.2) as bona fide m6A-binding proteins during parasite blood-stage development. Interaction proteomics reveal that PLYTH.2 associates with the translation machinery, including multiple subunits of the eukaryotic initiation factor 3 (eIF3) and poly(A)-binding proteins. Furthermore, functional knockdown coupled with ribosome profiling show that PLYTH.2 is essential for parasite survival and reveal this protein as a repressor of mRNA translation. Altogether, these data reveal an m6A-mediated mechanism controlling protein synthesis in a unicellular eukaryotic pathogen.

7 Adenine DNA methylation, and genome architecture in Trypanosoma brucei

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Trypanosoma brucei is a commonly transmitted parasite that colonizes the human urogenital tract causing infections that range from asymptomatic to highly inflammatory. Recent works have highlighted the importance of histone modifications in the regulation of transcription and parasite pathogenesis. However, the nature of DNA methylation in the parasite remains unexplored. Using a combination of immunological techniques and ultra-high performance liquid chromatography (UHPLC), we analyzed the abundance of DNA methylation in strains with differential pathogenicity demonstrating that N6-methyladenine (6mA), and not 5-methylcytosine (5mc), is the main DNA methylation mark in T. brucei. Genome-wide distribution of 6mA reveals that this mark is enriched at intergenic regions, with a preference for certain super-families of DNA transposable elements. We show that 6mA in T. brucei is associated with silencing when present on genes. Interestingly, bioinformatics analysis revealed the presence of transcriptionally active or repressive intervals flanked by 6mA-enriched regions and results from chromatin conformation capture (3C) experiments suggest these 6mA flanked regions are in close spatial proximity. These associations were disrupted when parasites were treated with the demethylation activator ascorbic acid. This finding revealed a new role for 6mA in modulating 3D chromatin structure and gene expression in this divergent member of the Excavata.

8 The Toxoplasma m6A epitranscriptome

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The most abundant internal mRNA modification is the N6-methylation of adenosines, termed the m6A mark. Over the course of the last decade, the m6A mark has come to the fore as a newly appreciated layer of post-transcriptional gene regulation, being implicated in nearly all facets from mRNA maturation to post-transcriptional control and turnover. Due to the conceptual similarities with epigenetics, the content and dynamic profile of methylated mRNA has been dubbed part of the epitranscriptome. Similarly, the regulatory machinery that deposits, recognizes, and removes the methyl marks are respectively referred to as m6A writers, readers, and erasers. Although the machinery surrounding the m6A mark appears to be essential for parasite viability it yet has not previously been investigated. Here, we show that Toxoplasma mRNAs are indeed modified with the m6A. We also tag each conserved member of the writer complex and have discovered putative accessory proteins contained within. We performed m6A-enrichment RNAseq, termed MeRIP-seq, to uncover the epitranscriptome in tachyzoites and parasites undergoing bradyzoite differentiation. The mark is widespread across the transcriptome. We also have begun investigating two putative m6A readers, both of which are localized to the nucleus, providing insights into their potential functions.

9 The trypanosome Surface Glycoprotein mRNA is stabilized by an essential unconventional RNA-binding protein

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Salivarian trypanosomes cause human sleeping sickness and economically important livestock diseases, transmitted by Tsetse flies and replicating extracellularly throughout the life cycle. The surface of “bloodstream forms”, which live within mammals, is coated by a monolayer of a Variant Surface Glycoprotein (VSG). Switching the expressed VSG gene enables the parasites to evade adaptive immunity. Adequate levels of VSG expression - 10% of total protein and 7% of mRNA - are partially attained through very active RNA polymerase I transcription and efficient mRNA processing, but a long mRNA half-life is also essential. Up until now, the mechanism by which VSG mRNA stability is maintained was unknown. Here, we apply an RNA-protein crosslink, RNA pulldown, and shotgun proteomics approach to identify proteins bound to VSG mRNA in Trypanosoma brucei. We find that an F-box protein, CF82, binds specifically to VSG mRNAs and is absolutely required to maintain VSG mRNA levels. CF82 in turn binds to a stabilizing complex that recruits poly(A) binding protein. VSG expression is essential not only for antigenic variation but also for cell division. Correspondingly, depletion of CF82 causes loss of VSG mRNA, cell cycle arrest, dramatic morphological abnormalities and trypanosome death. Since in these organisms the fate of mRNAs is largely influenced by RNA-binding proteins, we envision that this kind of approach will provide a platform for investigating RNA in vivo regulation.

10 TbMYND1, a novel mRNA stabiliser that controls trypanosome migration through the tsetse

Aitor Casas-Sanchez1,2,1, Lara Lopez-Escobar1, Aryan Zardkoohi-Burgos1, Cindy Cansado-Utrilla1, Lee Haines1, Simon Wagstaff1, Michael Lehaney1, Jannah Shamsan2, Samuel Dean3, Alistair Darby4, Neil Hall5, Derrick Robinson6, Laurence Wilson2, Pegine Wala4, Alvaro Acosta-Serrano1 1) Liverpool School of Tropical Medicine, UK; 2) University of York, UK; 3) University of Warwick, UK; 4) University of Liverpool, UK; 5) Earlham Institute, UK; 6) University of Bordeaux, France.
The journey of Trypanosoma brucei in the tsetse involves migration to several organs. This process occurs in a time manner and it is accompanied by a series of parasite developmental changes. Procylic trypanosomes initially establish an infection in the fly’s midgut, followed by proventricular colonization and then maturation in the salivary glands (SG), where differentiation from epimastigotes into metacyclic trypomastigotes occurs.

To identify T. brucei genes involved with life cycle progression in the tsetse, we compared the transcriptome profiles of proventricular trypanosomes from a fly-transmissible strain and a mutant strain unable to infect SG. We found >700 up-regulated transcripts in the fly-transmissible strain. The top hit was identified as a conserved hypothetical protein across kinetoplastid organisms, containing a predicted MYND (Myeloid, Nervy and DEAF-1) zinc-finger domain in the C-terminus. While overexpression of TbMYND1 restored infectivity of SG in the impaired strain, CRISPR-Cas9 deletion of TbMYND1 in a fly-transmissible strain resulted in a motility phenotype in vitro and in a very poor infectivity of the fly's midgut.

RBP6 is an RNA-binding protein essential for trypanosome differentiation in the tsetse. To investigate a possible interaction between the TbMYND1 and RBP6 pathways, we knocked out RBP6 using CRISPR-Cas9. RBP6 KO cells showed normal motility in vitro and infected both the midgut and the proventriculus at comparable levels with the parental cells. However, they failed to produce epimastigotes in the proventriculus and consequently did not infect SG. Pull-down assays showed that TbMYND1 interacts with a range of hypothetical proteins, most of them containing zinc-finger domains, including another MYND protein (TbMYND2). Furthermore, TbMYND1 is associated with >150 transcripts with products involved in flagellar attachment and cell cycle.

We propose that while RBP6 controls parasite differentiation at specific tsetse organs, TbMYND1 regulates trypomastigote migration through the fly.

11 Telomere maintenance in response to DNA damage in malaria parasites Jake Reed1,2,3, Laura Kirkman1,6, Christopher Mason2,3,4,5, Kirk Deitsch1 1) Department of Microbiology and Immunology, Weill Cornell Medical College, New York, New York; 2) Department of Physiology and Biophysics, Weill Cornell Medical College, New York, New York; 3) HH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Cornell Medical College, New York, New York; 4) Feil Family Brain and Mind Research Institute, Weill Cornell Medical College, New York, New York; 5) WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medical College, New York, New York; 6) Department of Internal Medicine, Division of Infectious Diseases, Weill Cornell Medical College, New York, New York.

Malaria caused by the protozoan parasite Plasmodium falciparum remains a major cause of morbidity and mortality within the developing world. These parasites have evolved a unique chromosome structure that includes the positioning of large, multicopy variant antigen gene families within the subteloemer regions of the parasite’s chromosomes, flanked by a highly conserved core genome and the telomere repeats at the chromosome ends. Recent work has implicated chromosome end stability and the repair of DNA breaks through telomere healing as poten drivers of variant antigen diversification, thus associating basic mechanisms for maintaining genome integrity with aspects of host-parasite interactions. Here we applied long-read sequencing technology to precisely examine the dynamics of telomere addition and chromosome end stabilization in response to double strand breaks within subteloemer regions. We observed that the process of telomere healing induces the initial synthesis of telomere repeats with a 2.25 fold increase in length as compared to non-healed ends. However once stabilized, these newly created telomeres appear to function normally, eventually returning to a length typical of intact chromosome ends. These results parallel recent observations in humans, suggesting an evolutionarily conserved mechanism for chromosome end repair.

12 Programmed DNA elimination in parasitic nematodes Jianbin Wang1,2, Giovana Veronezi1, Maximn Zagoskin1,2, Eileen O'Toole3, Richard Davis1 1) University of Colorado School of Medicine; 2) University of Tennessee at Knoxville; 3) University of Colorado.

Genomes rarely change. However, a few organisms undergo a wholesale genome change called DNA elimination, a programmed process that eliminates specific genomic sequences from the genome. We have shown that 18% of the genome of the parasitic nematode Ascaris is eliminated in somatic cell lineages during the third through fifth cleavages (4 to 16 cell stage), while the germline genome remains intact. Both specific repetitive and unique sequences (including ~1000 genes) are lost during the elimination process that forms the somatic genome. The DNA elimination events are identical in all individuals and in five distinct cell lineages. The eliminated genes are primarily expressed in the Ascaris germline and early embryo. Comparative genome analysis of DNA elimination in several other parasitic nematodes (A. lumbricoides, Toxocara, and Parascaris) demonstrated that they also eliminate 1,000-2,000 genes (5-10% of their genes). Overall, our data suggest that DNA elimination in parasitic nematodes is an essential, irreversible mechanism for silencing a subset of germline and early embryo expressed genes in somatic tissues. Comprehensive chromosome analysis of DNA elimination in Ascaris demonstrated that 72 DNA breaks occur and all 24 germline chromosome ends undergo remodeling through subteloemer DNA breaks, loss of terminal sequences, and healing of new chromosome ends by de novo telomere healing. Eliminated DNA is incorporated into micronuclei during telophase of a DNA elimination mitosis and the DNA is eventually degraded in autophagosomes. Worm specific Argonautes are on condensed chromosomes during elimination mitoses and are present either on chromosomes that will be retained (WAGO-2) or eliminated (WAGO-3). We hypothesize that the Argonautes and/or their small RNAs contribute to DNA elimination. Ascaris has holocentric chromosomes with multiple centromeric/kinetochore regions distributed along the length of the chromosome in the gametogenic germline. Ascaris holocentric chromosomes are dynamic. During DNA elimination, only those chromosome regions to be retained exhibit extensive deposition of the centromeric histone CENP-A and are holocentric; chromosome regions destined for elimination have lost CENP-A and are not segregated. Thus, both specific sites for DNA breaks and changes in CENP-A localization on the chromosomes are involved in a concerted mechanism to define portions of chromosomes for retention or elimination in programmed DNA elimination.

13 TcHTE expression is regulated by intracellular heme levels in Trypanosoma cruzi Evelyn Tevere1,2, Cecilia Beatriz Di Capua1,2, Julia Alejandra Cricco1,2 1) Instituto de Biología Molecular y Celular de Rosario – CONICET; 2) Facultad de Ciencias Bioquímicas y Farmacéuticas - Universidad Nacional de Rosario.

Trypanosoma cruzi is a heme auxotrophic organism and rely on the acquisition of this cofactor from their mammalian host or insect vector. Inside of the insect vector, T. cruzi may be in contact with hemoglobin (Hb) and heme derived from blood digestion. In vitro, T. cruzi epimastigotes can use both hemin and Hb as a heme source, which are internalized via different pathways. However, little is known about the use of Hb as a heme source.

TcHTE (T. cruzi Heme Transport Enhancer) is a protein belonging to the Heme Response Gene family. This protein is involved in heme uptake, probably being part of a heme transporter and controlling this activity. TcHTE is expressed mainly in the replicative life cycle stages of the parasites and it is localized to the flagellar pocket in epimastigotes.

In this work, we studied the role of TcHTE in heme homeostasis in Hb-supplemented parasites.

Conversely to the growth and morphology alterations in parasites supplemented with high hemin concentrations, epimastigotes tolerate high Hb concentrations in the medium and exhibit a typical morphology in these conditions. At mRNA and protein level, TcHTE is higher in heme-starved parasites and decreases when hemin or Hb are added to the medium. However, the response is less severe when Hb is used as heme source.
Surprisingly, epimastigotes that overexpress rTcHTE.His-GFP incubated in Hb-supplemented medium have a significantly higher intracellular heme compared to control epimastigotes; as previously reported in hemin-supplemented parasites. In addition, rTcHTE.His-GFP does not change its localization in Hb-supplemented parasites.

We concluded that T. cruzi is able to sense intracellular heme levels and regulates TcHTE expression according to it, independently of the heme source (hemin or Hb). Based on these and our previous results, we propose two complementary models of heme uptake in T. cruzi. In the first one, Hb is endocytosed and internally degraded. In the second one, Hb is externally degraded by parasite proteases and released heme enters the cell via TcHTE. The second model would explain why over-expression of rTcHTE.His-GFP produces an increase in the intracellular heme in Hb-supplemented parasites. In both models, once the parasite satisfies its heme requirements, TcHTE expression (and therefore heme transport) decreases.

14 Rhodopsin regulates lysophosphatidic acid induced macropinocytosis in *Entamoeba histolytica* Achaia Apte1, Maria Manich2, Elisabeth Labruyère3, Sunando Datta4 1) Indian Institute of Science Education and Research, Bhopal, India; 2) Institut Pasteur, Paris, France; 3) Institut Pasteur, Paris, France; 4) Indian Institute of Science Education and Research, Bhopal, India.

*Entamoeba histolytica* is a unicellular enteric protozoan responsible for variety of gastrointestinal pathologies. Amoebic trophozoites cause pathogenesis by migration, adhesion, phagocytosis, and macropinocytosis. These processes require dynamic changes in the cytoskeleton. Rho family GTPases, a major class of molecular switches, are activated by specific Guanine nucleotide Exchange Factors upon stimulation by various extracellular stimuli and play an indispensable role in controlling cytoskeleton remodelling.

In *E. histolytica*, there are 17 Rho GTPases harbouring a unique insert helix which confers Rho specific signalling events (Zong et al., 2001). We have investigated the cellular function of Rho5 and Rh01, belonging to same clade with 93% sequence identity. Amoebic Rhos and Rh01 translocated from cytosol to plasma membrane and endomembranous compartments when stimulated with LPA, a bioactive phospholipid known to activate Rho subfamily members (Fleming et al., 1996). Similar observations were made when starved trophozoites were replenished with serum. Our findings were strengthened when FRAP (Fluorescence recovery after photobleaching) study showed that LPA treated cells show 30% faster recovery of Rh05 when compared to serum starved cells. PDGF, a growth factor used for Rac activation, was unable to translocate Rh05, indicating that the GTPase belongs to Rho subfamily. This observation was further established by in vitro effector binding assay using mammalian Rho Binding Domain of the Rho effector protein Rhotekin, which binds specifically to the GTP-bound form of Rho subfamily (Van Leeuwen et al., 2015). Pulldown activation assay with mRBD confirmed Rhos activation following both LPA and serum treatment. LPA stimulation enhanced the macropinocytosis efficiency of trophozoites in a Rho5 dependent manner.

To probe the involvement of PI3K pathway in activation of Rhos upon LPA stimulation, we treated cells with wortmannin and observed that translocation of Rh05 was abrogated. Similar observations were made when FRAP studies were performed after wortmannin treatment, the recovery of Rh05 slowed down by 20%. We also observed that Rh05 overexpressing cells showed a marked reduction in their motility as measured through video microscopy followed by track analysis. Interestingly, earlier studies have shown cell migration and macropinocytosis to be antagonistic process (Veltman et al., 2014; Chabaud et al., 2015). We are currently studying the correlation between these two processes in *Entamoeba histolytica*, if any.

15 A unique Na+-Pi cotransporter in *Toxoplasma* plays key roles in phosphate import and control of parasite osmoregulation Beejan Asady1, Claudia Dick1, Karen Ehrenman1, Julia Romano1, Tejram Sahu1, Isabelle Coppens1 1) Johns Hopkins.

Inorganic ions such as phosphate are essential nutrients required for a broad spectrum of cellular functions and regulation. During infection, pathogens must obtain inorganic phosphate (Pi) from the host. Despite the essentiality of phosphate for all forms of life, how the intracellular parasite *Toxoplasma gondii* acquires Pi from the host cell is still unknown. In this study, we demonstrated that *Toxoplasma* actively internalizes exogenous Pi by exploiting a gradient of Na+ ions to drive Pi uptake across the plasma membrane. The Na+-dependent phosphate transport mechanism is electrogenic and functionally coupled to a ciparigin-sensitive Na+-H+-ATPase. *Toxoplasma* expresses one transmembrane Pi transporter harboring PHO4 binding domains that typify the PiT family. This transporter named TgPiT, localizes to the plasma membrane, the inward buds of the endosomal organelles termed VAC, and many cytoplasmic vesicles. Upon Pi limitation in the medium, TgPiT is more abundant at the plasma membrane. We genetically ablated the PiT gene, and ΔTgPiT parasites are impaired in importing Pi and synthesizing polyphosphates. Interestingly, ΔTgPiT parasites accumulate 4-times more acidocalcisomes, storage organelles for phosphate molecules, as compared to parental parasites. In addition, these mutants have a reduced cell volume, enlarged VAC organelles and defects in Ca2+ stores, and are unable to regulate their cytosolic Na+ concentration and pH. Overall, these mutants exhibit severe growth defects and have reduced acute virulence in mice. In survival mode, ΔTgPiT parasites upregulate several genes, including those encoding enzymes that cleave or transfer phosphate groups from phosphomonoesters, transporters and ions exchangers localized to VAC or acidocalcisomes. Taken together, these findings point to a critical role of TgPiT for Pi supply for *Toxoplasma* and also for protection against osmotic stresses.

16 *Toxoplasma gondii* Resistance to Interferon-gamma Dependent Killing Requires a Parasite Oxygen Sensing Protein Charlotte Cordonnier1, Msano Mandalasi2, Christopher West2, Ira Blader1 1) Department of Microbiology and Immunology, University at Buffalo; 2) Department of Biochemistry and Molecular Biology, University of Georgia.

*T. gondii* is an obligate intracellular parasite that is an important opportunistic infection in AIDS patients and other immunocompromised individuals. Human infection typically occurs through the ingestion of contaminated food or water. After breaching the intestinal epithelial barrier, the parasite spreads to a large variety of other organs such as the brain, eyes and placenta. During its journey through the host, *Toxoplasma* is exposed to diverse O2 tensions and must adapt to changes in O2 availability for its survival and virulence. Cytoplasmic prolyl 4-hydroxylases (PHDs) are O2 sensors that regulate cellular responses to changes in O2 availability. *Toxoplasma* expresses two PHDs. One of them, TgPhyA hydroxylates Skp1, a subunit of the E3-SCF ubiquitin ligase complex. In *vitro*, TgPhyA is important for growth at low O2 levels. However, no studies have focused yet on the *in vivo* importance of TgPhyA. Using a ype II ME49 *Toxoplasma* PhyA knockout, we report that PhyA is important for *Toxoplasma* virulence and brain cyst development. While TgPhyA mutant parasites can infect the gut they are unable to efficiently disseminate to peripheral tissues. We further find that this is due to a requirement for TgPhyA to resist IFN-gamma dependent killing within inflammatory monocytes. To our knowledge, this represents the first example of a pathogen O2-sensing pathway that contributes to virulence by acting to disable a key immune defense pathway.

17 *Plasmodium falciparum* gametocyte diversity prior to and after malaria treatment with an artemisinin-based combination therapy Mary Oboh1, Kolapo Oyebola2, Olumide Aijibola3, 3, Alfred Amambua-Ngwa1 1) Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine; 2) National Institute of Health, MarylandLand USA; 3) First Technical University, Ibadan, Oyo state Nigeria.

Malaria transmission relies on the parasite’s ability to produce the sexual stages (gametocytes) that are picked up by the mosquitoes to produce the infective transmissible stage: sporozoites. Immature sexual gametocytic stages are usually sequestered in blood vessels but find their way to peripheral blood. While these mature gametocytes are mostly not detected by microscopy, they contribute to transmission. Hence, understanding the dynamics of sub-microscopic infections within the human infectious reservoir is relevant for strategies towards malaria elimination. In line with this, we have studied the diversity of four *P. falciparum* gametocyte genes (Pfs230, Pfs25, PfG377 and Pfgvd1) from malaria infected individuals before (day 0) and after artemisinin combination
treatment (day 1) from south-western Nigeria. Newly designed amplification and sequencing primers were used to amplify the fragments of all four genes and sequenced bi-directionally in the SeqStudio capillary electrophoresis Unit at MRCG. Following careful examination of the electropherogram and base calling, derived consensus sequences were aligned against the reference for variant determination. Of the total participants recruited for this study, 80 were from day 0 while the others (40) were day one post-treated subjects from the day 0 group. We observed high haplotype diversity in the Pfg377 gene both in pre- and post-treated individuals. Approximately 20% of pre and post-treated haplotypes were similar for the Pfg377 gene. Directional selection was detected with the P. falciparum gametocyte development gene 1 (Pfgdv1) within pre and post treated isolates. The detection of similar haplotypes of the Pfs230 in pre- and post-treated individuals indicate the transmission ability of similar parasite genotype after treatment. From this preliminary data, a population appraisal of pre- and post chemotherapy and chemoprophylaxis rates of gametocytemia in south western Nigeria and the diversity of these loci relevant for transmission.

18 Whole-genome sequencing of *Crithidia*-like parasites isolated from relapsing human visceral leishmaniasis in Brazil Taïlla Takahashi1, Luana Rogerio1, Nayore Takamiya1, Amelia Jesus2, Roque Almeida2, Jose Ribeiro3, Joao Silva4, Sarah Forresters5, Daniel Jeffares5, Sandra Maruyama1 1) Federal University of Sao Carlos, UFSCar, Sao Carlos, SP, Brazil; 2) Federal University of Sergipe, Aracaju, SE, Brazil; 3) National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD, USA; 4) Oswaldo Cruz Foundation, Fiocruz, Rio de Janeiro, RJ, Brazil; 5) University of York, York, UK.

Leishmaniasis is caused by *Leishmania* species. Sporadic reports of infections (or co-infections) with apparently monoxenous trypanosomatids (one-host parasites) have been described in human cases of cutaneous (CL) and visceral leishmaniasis (VL) in last decades. The post-genomic era has increased these reports by DNA sequencing of parasites from clinical samples. However, it is still unknown the implications of these "unusual" infections in clinical outcomes and epidemiology. Are they occasional findings or evidence for enzootic human parasites? Previously, we identified a non-*Leishmania* parasite (LVH60 strain) in clinical isolates from a fatal case of VL in Sergipe, northeast Brazil. Phylogenomics showed that it is closely related to monoxenous non-pathogenic species *Crithidia fasciculata*, being referred here as *Crithidia*-like. By analysing SSU/rRNA sequences from other clinical isolates of VL from the same endemic area, we found that most of them typed as *Crithidia*-like rather than *Leishmania*. Here, we performed whole-genome sequencing analysis of four samples of *Crithidia*-like parasites isolated from two VL cases characterized by treatment-refractoriness and recidivism. For this, genomic DNA of clones from each clinical isolate were sequenced in Illumina platform and one clone (LVH60a-c1) also sequenced by Oxford Nanopore Technology (ONT). Illumina sequencing yielded a genome with coverage ranging from 20X to 40X and ONT sequencing generated 2.9 Gb of long-reads data with N50 read length of 28.4 Kb (30X depth coverage), which enabled an initial genome assembly with 97 contigs. Polishing up genome assembly resulted in 38 contigs with a predicted haploid genome size of 34.4 Mb. Genome alignment between LVH60a-c1 and *C. fasciculata* presented roughly 92% of nucleotide identity and among *Crithidia*-like clones approximately 98%, suggesting that they do not belong to *C. fasciculata*. Ploidy was evaluated by chromosome copy number estimation using read depth. *Crithidia*-like contigs #12 (479,386 bp) and #36 (2,180,050 bp) were inferred to be trisomic. A total of 11,290 coding sequences were deduced from 38 contigs. The genome has currently been annotated to gain insights about this newly identified parasite involved in human VL cases. These findings open a research path to address epidemiological questions about vectors, reservoirs, distribution patterns and the reassessment of leishmaniasis cases in Brazil. Financial Support: FAPESP, CNPq and CAPES.

19 Utilizing interspecies comparisons between *Toxoplasma gondii* and *Hammondia hammondi* to identify mechanisms regulating stage conversion Sarah Solok Borrelli1, JP Dubey 2, Jon Boyle 1 1) Department of Biological Sciences, Kenneth P. Dietrich School of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania; 2) Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

In eukaryotic parasites stage conversion events are associated with dramatic changes in gene expression and are critical for pathogenesis and transmission. In *Toxoplasma gondii*, despite extensive work profiling genome-wide transcriptional changes during stage conversion, only a handful of genes that are core regulators of this process have been identified. In eukaryotic parasites, pre-cyst formation from these extracellular stages is a major challenge due to the magnitude of transcriptional changes that occur during both natural and laboratory-induced cytokinesis. With this challenge in mind, we have exploited the *Toxoplasma gondii/Hammondia hammondi* comparative system in order to identify regulators of cytokinesis. This system is ideal because despite having extensive genomic similarity to *T. gondii* this parasite species has key differences in life stage progression. Specifically, *H. hammondi* displays differences in the timing and efficiency of tissue cyst formation and responds poorly to tissue cyst-inducing stimuli (such as alkaline pH). Using transcriptome data from critical developmental timepoints for both species and in response to alkaline pH, we have exploited the *Toxoplasma gondii/Hammondia hammondi* comparative system in order to identify regulators of cystogenesis. This system is ideal because despite having extensive genomic similarity to *T. gondii* this parasite species has key differences in life stage progression. Specifically, *H. hammondi* displays differences in the timing and efficiency of tissue cyst formation and responds poorly to tissue cyst-inducing stimuli (such as alkaline pH). Using transcriptome data from critical developmental timepoints for both species and in response to alkaline pH, we have identified a small number of candidate genes (~20) that we hypothesize function as transcription factors and/or nucleic acid binding proteins that regulate stage conversion in both species, some of which have already been implicated in regulating stage conversion such as BFD1 and AP2X1-9. Genetic ablation of one of the so far uncharacterized candidates, the putative RNA-binding protein TgVEG_311100, significantly reduced the number of tissue cysts formed *in vitro*, and the tissue cysts that did form had an impaired ability to interact with a lectin which specifically binds tissue cyst walls. In addition to a strong defect in tissue cyst formation, ΔTgVEG_311100 parasites also had a muted transcriptional response when exposed to cyst-inducing conditions, making it a clear member of the emerging group of pre-cyst development genes. 

20 Whole genome capture enrichment sequencing of enteroparasite *Cryptosporidium* directly from patient stool samples Asis Khon1, Eliza Carneiro Alves Ferreira1, Andrew J. Oler2, Michael E. Grigg1 1) Molecular Parasitology Section, Laboratory of Parasitic Diseases, National institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; 2) Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

*Cryptosporidium* is the 2nd leading cause of severe diarrhea and mortality in young children and infants in Africa and southern Asia. So far, ~30 distinct species of *Cryptosporidium* are known to cause severe to moderate infections in humans, of which *C. hominis* and *C. parvum* are the major causative agents. Very few low-resolution typing markers exist to genotype and/or diagnose *Cryptosporidium*. Hence, there is an immediate need to develop high resolution genetic markers to genotype *Cryptosporidium* parasites at whole genome scale directly from stool samples and to understand the potential for zoonotic transmission and the evolution of these protozoan parasites. We developed SureSelect capture enrichment sequencing (CES) on an Illumina 2500 platform to understand the global population genetic structure of *Cryptosporidium*. Our platform has 75,000 probes that cover ~95% genome in order to hybridize *Cryptosporidium* DNA directly from stool samples. Initial spooling experiments show that CES can amplify 30% of the genome using only 0.01ng *Cryptosporidium* gDNA spiked into 200ng of gDNA from host stool. Our initial analysis also showed that CES can be applied very specifically and sensitively not only to amplify distantly related species including *C. meleagris* but also to detect mixed infection of *Cryptosporidium* spp. Whole genome sequence data from CES enabled us to analyze genetic diversity using PopNet, PCA and POPSICLE software suites and to capture the population structure of *Cryptosporidium* spp., which will be presented. Thus, our data revealed that CES is a culture independent robust methodology to sequence whole genome of a pathogen directly from clinical specimen.

21 Exploring the evolution and adaptive role of mosaic aneuploidy in clonal *Leishmania donovani* populations, with high throughput single cell genome sequencing Malgorzata A Domagalska1, Gabriel Negreira1, Pieter Monsieurs1, Hideo Imamura1, Ilse Maes1, Frederik Van den Broeck1, Yvon Sterkers2, Jean-Claude Dujardin1 1) Institute of Tropical Medicine, Molecular Parasitology Unit, Antwerp, Belgium; 2) University of Montpellier 1, UFR Médecine, Laboratoire de Parasitologie-MycoLigien Montpellier, France.
emerging from late trophozoites during the temperature treatment, showing a previously unknown potentially reactive state with high degree of antigenic
upregulation of AP2-G, the master regulator of sexual development in this small fraction of parasites. We also identified a rare population of parasites
parasites matured with a small fraction of parasites expressing genes related to the process of gametocytogenesis. Interestingly, temperature treatment results
~30 nm) and temporal resolution (t = 10 ms). The length of gained trajectories is mainly limited by the shape and size of trypanosomes (approx. 50 µm2 in the
cyto-compatible hydrogel embedding. Building on this work, we are now able to track VSG dynamics on living trypanosomes at high spatial (localization precision
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field forces
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Relatedness and mutation shape the genomic diversity of recurrent Plasmodium vivax infection Aliou Dia1, Catherin Jett1, Simon Trevino1, Cindy Chu3,4, Kanlaya Srirawat4, Timothy Anderson2, Francois Nosten3,4, Ian Cheeseman1,1) Host-Pathogen Interaction Program, Texas Biomedical Research Institute, Texas, USA; 2) Disease Prevention and Intervention Program, Texas Biomedical Research Institute, Texas, USA; 3) Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK; 4) Shoklo Malaria Research Unit, Mahidol University, Mae Sot, Thailand.

Infections with the malaria parasite Plasmodium vivax can recur due to either relapse from liver stage hypnozoites, recrudescence from blood stage
parasites or reinfection by an infected mosquito bite. We explored genomic variation across recurrent P. vivax infections using a novel single cell sequencing
protocol optimized for low paraitaemia infections, obtaining near-complete coverage of the parasite genome from individual cells. Genome sequences from 441
single parasite genomes from across 11 patients sampled during multiple febrile episodes confirm that in this setting recurrences are predominantly from
identical or closely related genotypes and likely resulted from liver stage relapse or blood stage recrudescence. We perform conservative identification of
single parasite genomes from across 11 patients sampled during multiple febrile episodes confirm that in this setting recurrences are predominantly from
ploidy over continuous mitotic events is a paradigm for most higher eukaryotes. Defects in chromosome segregation and/or replication can lead to aneuploidy, a condition usually associated with deleterious effects. However, in Leishmania, a Protozoan parasite, aneuploidy is an ubiquitous feature, where variations of chromosome copy number (CCN) in the cell population represent a mechanism of gene expression adaptation, possibly impacting phenotypes. Moreover, even in clonal populations, individual CCN varies significantly between single cells, a phenomenon named mosaic aneuploidy (MA). Until recently, the only technique available to study MA in Leishmania was FISH which, despite its single-cell resolution, only allows somy assessment of few chromosomes, not providing information about the complete karyotype of single-cells. To overcome these limitations, we used here for the first time a high-throughput Single-Cell Genome Sequencing (SCGS) pipeline (Chromium Single Cell CNV – 10X GenomicsTM) to analyze the complete karyotype of thousands of individual promastigotes from 2 clonal L. donovani strains. The strains BPK282 and BPK081 were submitted to SCGS at 21 and 7 passages after cloning and
revealed 220 and 119 different karyotypes respectively. A network analysis was made to gain insights on the structure of karyotype variation in both populations. In BPK282, most karyotypes were found to be linked to each other by changes in somy of single chromosomes, which allowed us to propose a hypothesis of stepwise MA evolution, starting from a founder and yet highly aneuploid karyotype. In BPK081, the structure of the network was very different, with 3 dominant karyotypes differing between each other by CCN changes in 8 to 10 chromosomes. We propose the hypothesis that in this strain, in which the founder karyotype had an almost completely disomic genome, MA was initiated by big 'jumps' through rapid and multiple CCN changes. For strain BPK282, a second type of analysis was done in the context of drug resistance. We found indeed that aneuploidy patterns that were previously described by Bulk Genome Sequencing as emerging during early phases of drug resistance selection were already present in single karyotypes in the SCGS data, suggesting a (pre-)adaptive role of MA. Our results provide an unprecedented insight on the extent and dynamics of MA and pave the way for further functional studies.

Preliminary data are reported in https://www.biorxiv.org/content/10.1101/2020.03.05.976233v1

22 Single-molecule dynamics of the trypanosomes’ VSG coat Marie Schwebs1, Torsten Paul2, Marius Glogger1, Philip Kollmannsberger2, Markus Engstler1, Susanne Fenzi1) Department for Cell and Developmental Biology, Biocenter, University of Wuerzburg, Germany; 2) Center for Computational and Theoretical Biology, University of Wuerzburg, Germany.

Trypanosoma brucei expresses a dense coat of GPI-anchored variant surface glycoproteins (VSGs). The fluidity of this coat is fundamental for the evasion of the host’s immune system and thus for the survival of the parasite. So far, the VSG dynamics on living trypanosomes has been studied at the micron and second scale for the whole ensemble.

In this project, we want to elucidate with single-molecule fluorescence microscopy the dynamics of individual VSGs in relation to the flagellar pocket, the sole site for endo- and exocytosis. For this purpose, we have recently introduced super-resolution imaging of intrinsically fast-moving flagellates based on cyto-compatible hydrogel embedding. Building on this work, we are now able to track VSG dynamics on living trypanosomes at high spatial (localization precision ~30 nm) and temporal resolution (t = 10 ms). The length of gained trajectories is mainly limited by the shape and size of trypanosomes (approx. 50 µm2 in the focal plane). Therefore, we use an image-based analysis pipeline inspired by Hoze and Holman [Biophys. J., 2014] to make reliable statements about local drift field forces \( u(x) \) and the diffusion tensor \( D(x) \) for each surface point. The information is gained from a large number of short trajectories and will be presented in directed motion and diffusion maps.

23 Single-cell RNA sequencing reveals cellular heterogeneity, stage transition and antigenic variation during stress adaptation in synchronized Plasmodium falciparum Mukul Rawat1, Ashish Srivastava1, Shreya Johri2, Ishaan Gupta2, Krishnanal Karmodya1) Indian Institute of Science Education & Research, Pune, India; 2) Indian Institute of Technology Delhi, India.

The malaria parasite has a complex life cycle exhibiting phenotypic and morphogenic variations in two different hosts by existing in heterogeneous developmental states. To investigate this cellular heterogeneity of parasite within the human host, we performed single-cell RNA sequencing (scRNA-seq) of 11822 synchronized Plasmodium cells in control and under temperature treatment conditions (phenocopying the cyclical bouts of fever). Using the Malaria Cell Atlas as a guide, we identified fine-grained gene expression signatures in 9 subtypes of the parasite distributed across known intra-erythrocytic stages. Pseudotime analysis revealed a developmental switch-like behaviour as the parasite moved from early to late trophozoite stage of intra-erythrocytic development. Upon commitment to late trophozoite stage the parasite immediately branched to form a pool of stress-adapted trophozoites while the remaining parasites matured with a small fraction of parasites expressing genes related to the process of gametocytogenesis. Interestingly, temperature treatment results in upregulation of AP2-G, the master regulator of sexual development in this small fraction of parasites. We also identified a rare population of parasites emerging from late trophozoites during the temperature treatment, showing a previously unknown potentially reactive state with high degree of antigenic variation. We also developed a complementary online tool (http://sidd.info/synchronized_plasmodium_cell_atlas/) to explore single and multiple genes expression profiles under normal and temperature treatment conditions while exploring the same genes in the Malaria Cell Atlas simultaneously. Thus, our study reveals important insights into the cell-to-cell heterogeneity in the parasite population under the temperature treatment condition that will be instrumental towards mechanistic understanding of cellular adaptation and population dynamics in Plasmodium falciparum.

24 Relatedness and mutation shape the genomic diversity of recurrent Plasmodium vivax infection Aliou Dia1, Catherin Jett1, Simon Trevino1, Cindy Chu3,4, Kanlaya Srirawat4, Timothy Anderson2, Francois Nosten3,4, Ian Cheeseman1,1) Host-Pathogen Interaction Program, Texas Biomedical Research Institute, Texas, USA; 2) Disease Prevention and Intervention Program, Texas Biomedical Research Institute, Texas, USA; 3) Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK; 4) Shoklo Malaria Research Unit, Mahidol University, Mae Sot, Thailand.

Infections with the malaria parasite Plasmodium vivax can recur due to either relapse from liver stage hypnozoites, recrudescence from blood stage parasites or reinfection by an infected mosquito bite. We explored genomic variation across recurrent P. vivax infections using a novel single cell sequencing protocol optimized for low paraitaemia infections, obtaining near-complete coverage of the parasite genome from individual cells. Genome sequences from 441 single parasite genomes from across 11 patients sampled during multiple febrile episodes confirm that in this setting recurrences are predominantly from identical or closely related genotypes and likely resulted from liver stage relapse or blood stage recrudescence. We perform conservative identification of de novo mutations arising within individual patients to explore the dynamics of recurrent infections at single genome resolution. We leverage this catalogue of de novo mutations to identify mutational hotspots in the parasite genome. We find recurrently hit gene families involved in antigenic variation and transcriptional regulation highlighting the capacity for intrahost evolution.

25 TcAMPK: a cellular energy homeostasis hub regulator with unique characteristics in Trypanosoma cruzi Tamara Sternlieb1, Alejandra C. Schojett1,3, Patricia D. Genta1, Guillermo D. Alonso1,2) 1) Instituto de Investigaciones en Ingeniería Genética y Biología Molecular “Dr. Héctor N. Torres” - CONICYT; 2) Departamento de Fisiología, Biología Celular y Molecular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires; 3) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme involved in maintaining energy homeostasis in response to different stresses in many organisms. During the transition between the mammalian host and the insect vector, Trypanosoma cruzi, the causative agent of Chagas disease, faces different types of environmental fluctuations, all of which prompt the parasite to remodel its metabolism to adapt, survive and differentiate into the next stages
of its life cycle. Recently, it was shown that Trypanosoma brucei AMPK is involved in the differentiation from the bloodstream slender to stumpy stage and in surface protein expression changes in response to nutritional stress. This underscores the relevance of AMPK for parasite life cycle progression.

We identified four candidate genes for the AMPK subunits of T. cruzi (α1 and α2 catalytic subunits, β and γ regulatory subunits). The β and γ subunits are largely conserved in their domain structure relative to the mammalian orthologs. However, the alpha subunits show significant sequence, structure and evolutionary differences from the human counterparts. The presence of these subunits in T. cruzi epimastigotes was confirmed by RT-PCR. Western blot using a phospho-AMPKα specific antibody, mass spectrometry and by kinase activity assays using the specific AMPK substrate SAMS. TcAMPKα1 over-expressing epimastigotes showed a lower growth rate in basal culture conditions compared to the control, while α2 over-expression had the opposite effect. We found there is upregulation of AMPK activity under epimastigote starvation, which is prevented if glucose is present in the culture medium. Dorsochoromorph, a specific AMPK inhibitor, also inhibits T. cruzi AMPK. Each of these subunits could complement the ‘glucose dependent’ phenotype of S. cerevisiae conditional mutants lacking the respective subunit of the AMPK ortholog Snf1. Starvation and Monodansylcadaverine incorporation assays with AMPKs over-expressing parasites also showed a possible role of AMPK in autophagy. Finally, we explore the pathways in which TcAMPK may be involved using in silico analysis of putative substrates. Our results open the door to the study of the AMPK signaling pathway in Trypanosoma cruzi.

26 The malarial Stearyl-CoA desaturase is essential only for parasite late liver stage development Sunil Narwal1, Hadi Choudhary1, Satish Mishra1 1) CSIR-Central Drug Research Institute, Lucknow, India.

Apicomplexan parasite, Plasmodium replicate exclusively within host cells thus requires nutrients and fatty acids which are essential for membrane formation. One of the principal components of membrane is oleic acid and that is required for maintaining the membrane biophysical properties and fluidity. Malaria parasite Plasmodium is capable of modifying fatty acids and Stearyl-CoA Δ9-desaturase (SCD) is an enzyme, catalysing the synthesis of oleic acid by desaturation of stearic acid. Expression analysis revealed the presence of Scd transcripts across all Plasmodium life stages, with maximal expression in liver and blood stage schizonts. This likely point to its role in membrane biogenesis required during merozoite formation. Disruption of Scd in rodent malaria parasite P. berghei did not affect parasite blood stage propagation, mosquito stage development and early liver stage development. However, when Scd knockout (KO) sporozoites were inoculated intravenously or by mosquito bite in mice, they failed to initiate the blood stage infection. Immunofluorescence analysis with MSP1 and ACP antibodies of late stage liver stage exo-erythrocytic forms revealed that merozoite formations were impaired and did not show the branched apicoplast structures. RNA sequencing analysis of 55 h Scd KO EEFs revealed that attenuation at late developmental stage was associated with down regulation of genes central to general transcription, cell cycle, energy metabolism, fatty acid and merozoite formation. Further, C57BL/6 mice immunized with Scd KO parasites were protected against infectious sporozoites challenge. On the basis of these observations, we propose the development of genetically attenuated P. falciparum parasites as a pre-erythrocytic stage vaccine candidate.

27 Loss of TgLaforin, a glucan phosphatase, renders T. gondii tachyzoites unable to survive without glutamine Robert Murphy1,2, Animesh Dhar2, Ramon Sun1, Cortni Troublefield2, Corey Brizee2, Tiantian Chen1, Anthony Sina2, Matthew Gentry1 1) University of Kentucky, College of Medicine, Department of Molecular and Cellular Biochemistry, Lexington, KY; 2) University of Kentucky, College of Medicine, Department of Microbiology Immunology and Molecular Genetics, Lexington, KY.

The asexual cycle of T. gondii is defined by rapidly dividing tachyzoites during the acute infection, and bradyzoites within tissue cysts during the chronic phase. Bradyzoites accumulate starch-like glucose polymers known as amylpectin granules (AGs), which are notably absent in tachyzoites. Importantly, the T. gondii genome encodes the activities needed for starch (AG) turnover that are well characterized in plants. These include a glucan-water dikinase ("TgGWD": TgME49_214260) and a glucan phosphatase ("TgLaforin": TgME49_205290) which catalyze a cycle of direct glucan phosphorylation-dephosphorylation required for starch degradation by multiple amylases. It is not known, however, if T. gondii utilizes this cycle in AG degradation.

To verify the functions of TgLaforin and TgGWD, we expressed these proteins in Sf9 insect cells and confirmed that both functioned as predicted in cell-free assays. Both enzymes demonstrated the ability to specifically bind starch, exhibited their respective glucan phosphatase/kinase activities, and utilized mutually compatible carbon phospho-sites on glucose. To investigate the importance of AG phosphorylation in T. gondii, we generated a CRISPR/Cas9 knockout of TgLaforin. As expected, loss of TgLaforin did not result in tachyzoite growth defects under nutrient-replete conditions. However, despite the traditional view that AG metabolism is only relevant in bradyzoites, we found that the viability of TgLaforin-KO tachyzoites was completely dependent on the presence of glutamine, even under glucose replete conditions. In vivo infection with TgLaforin-KO tachyzoites resulted in attenuated virulence defined by both a delayed onset of acute symptoms and lower mortality in mice. Notably, TgLaforin-KO infected animals exhibited impaired cyst yields. Moreover, the ability of TgLaforin-KO cysts to establish a new infection following i.p injection was also compromised.

We are actively addressing how the loss of TgLaforin appears to have fundamentally altered intermediary metabolism in tachyzoites, a life cycle stage in which AGs are assumed to not be involved. In addition, we are directly addressing how the perturbation of starch metabolism is impacting the progression of the chronic infection in vivo.

28 Molecular and biochemical characterization of a phosphoglycerate kinase containing a Per-ARNT-Sim domain, from Trypanosoma cruzi. Maura Rojas-Pirela1, 2, Yossmayer Rondón Guerrero 1, 3, Andrea Delgado1, Ana Cáceres1, Paul Michel1, Juan Luis Concepción1, Wilfredo Quiñones1 1) Laboratorio de Enzimología de Parásitos, Departamento de Biología, Facultad de Ciencias, Universidad de Los Andes, Mérida 5101, Venezuela; 2) Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso 2373223, Chile; 3) Fundación Instituto Leloir and Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET). Av. Patricia Argentina, Buenos Aires CP, Argentina.; 4) Centre for Immunity, Infection and Evolution and Centre for Translational and Chemical Biology, School of Biological Sciences, The University of Edinburgh, The King's Buildings, Edinburgh EH9 3LF, United Kingdom.

The Per-ARNT-Sim (PAS) domains are a family of protein domains present in a wide variety of organisms, including parasites. The PAS domain is part of the structure of several proteins involved in diverse cellular processes, such as those related to adaptation to environmental conditions. The regulation of enzymatic activity, through the binding of small ligands, is among the main functions attributed to PAS proteins. Recently, a wide diversity of PAS proteins was identified in the genome of several kinetoplastids, however, until now few of these proteins have been characterized. In the present work, we characterized a phosphoglycerate kinase containing a PAS domain, in Trypanosoma cruzi (TcPAS-PGK). This PAS-PGK is an active enzyme of 58 kDa with a PAS domain located at its N-terminal side. We located it in the glycosomes of T. cruzi epimastigotes by two different approaches (differential centrifugation and selective permeabilization with digitonin), besides the presence of the protein in a fraction enriched in mitochondria. Two heterologous expression systems for TcPAS-PGK over-expressing parasites also showed a possible role of AMPK in autophagy. Finally, we explore the pathways in which TcAMPK may be involved using in silico analysis of putative substrates. Our results open the door to the study of the AMPK signaling pathway in Trypanosoma cruzi.
to concentration highly than 500 mM and 450 mM respectively. The presence of hemin and FAD exerts a stimulatory effect on the activity of PAS-PGKc, increasing the specific activity by 155%. This stimulation is not observed in the absence of the PAS domain. It can be concluded that the PAS domain has an important function in the modulation of the catalytic activity of the TcPAS-PGK. In addition, the PAS-PGK, through its PAS and PGK domains, could act as sensor of environmental conditions in the parasite.

29 Plasmodium protein export complex functions at both the parasite-host cell interface and within the parasite. Mikha Gabriela1,2, Dene Littler3, Cas Boshoven1, Brad Sleeks4, Benjamin Dickerman1, Paul Sanders1, Jamie Rossjohn3, Tania de Konin-Ward2, Brendan Crabb1,3,5, Paul Gilson1 1) Burnet Institute, Melbourne, Australia; 2) School of Medicine, Deakin University, Waurn Ponds, Australia; 3) Monash University, Melbourne, Australia; 4) Walter and Eliza Hall Institute, Melbourne, Australia; 5) University of Melbourne, Melbourne, Australia.

Blood stage Plasmodium parasite dedicates ~10% of its proteome to modify its host erythrocyte’s physiology. To reach the erythrocyte compartment, parasite-derived effector proteins need to be unfolded and translocated across an enveloping parasitophorous vacuole membrane (PVM). This is carried out by Plasmodium translocon of exported proteins (PTEX), a multiprotein complex consisting of a pore-forming protein EX2, structural protein PTEX150, and an AAA+ ATPase HSP101. The mechanism by which the effector proteins are recruited to PTEX for translocation has been a longstanding question. A current hypothesis is that the mature N-terminal amino acids of effector proteins, exposed after proteolytic cleavage within their PEXEL export motifs in the endoplasmic reticulum (ER), act as a recognition signal for HSP101 embedded in PTEX at the PVM. To explore this, we recombinationally expressed the putative substrate binding domain of HSP101 (HSP101NTD) and found that it did not specifically interact with a model effector protein containing the mature N-terminal sequence of PEXEL motif, despite several possible binding pockets founds in the crystal structure of HSP101NTD. In parallel, we expressed various effector reporter proteins in parasites containing mutated N-terminal amino acids that were to be exposed after PEXEL cleavage. One effector mutant was retained in the ER and microscopy surprisingly revealed the presence of HSP101 pool co-localised with this mutant protein, with the remainder localised with EX2 at the PVM. To examine whether this parasite resident HSP101 subpopulation was functional, we performed several biochemical experiments and found that HSP101 had already interacted with the mutant effector protein in the ER, independent of PEXEL motif processing. These data challenge the previous model where PTEX first recognises cargo at the PVM and indicated that cargo recruitment by PTEX may potentially begin in the ER without direct contribution of the PEXEL motif.

30 ROP16 facilitates type III Toxoplasma gondii cyst development through activation of STAT6. Joshua Kocjanowsk1, Anita Koshy1 1) University of Arizona.

ROP16 facilitates type III Toxoplasma gondii cyst development through activation of STAT6.

Toxoplasma gondii is an intracellular protozoan parasite that establishes a persistent infection of the central nervous system (CNS). CNS persistence, which depends on T.gondii converting from a fast replicating form, the tachyzoite, to a slow growing encysted form, the bradyzoite, underlies the parasite’s ability to reactivate to cause devastating neurologic disease in the immunocompromised. Most work on cyst development has focused on parasite genes that affect this transition, with less work done to define the host factors that influence the tachyzoite-bradyzoite switch.

Here we report that rhoptry protein 16 (ROP16), a highly studied effector protein that manipulates host cells in a T.gondii strain-specific manner, plays a previously unrecognized role in encystment in type III parasites. The two canonical encysting T.gondii strains, type II and type III, carry different alleles of ROP16. Both ROP16s translocate into the host cell nucleus and have a functional kinase domain, but only the type III allele (ROP16III) causes prolonged activation of STAT3, Sa, and 6. While a type I strain that lacked ROP16 showed a mild increase in encystment, a type III strain that lacked ROP16 (Illrop16) showed a marked decrease in cyst conversion in both human foreskin fibroblasts (HFFs) and primary murine neurons. This defect was complemented in trans by co-infection with wild type parasites, suggesting that ROP16III facilitates cyst development through manipulation of the host cell. Through studies using HFFs with STAT 3, Sa, or 6 knocked down and murine neurons lacking STAT6, we identified that efficient encystment is dependent on ROP16’s ability to activate STAT6. We also determined that, in vivo, parental type III parasites have a lower cyst-burden in STAT6 knockout mice compared to wild type mice. Current work is directed toward identifying what STAT6-dependent host cell genes drive this ROP16III effect.

31 Toxoplasma gondii GRAbs host cytosolic proteins by interacting with the host ESCRT machinery Yolanda Rivera-Cuevas1, Joshua Mayoral2, Louis M. Weiss2, Manlio Di Cristina3, Vern B. Carruthers2 1) University of Michigan, Ann Arbor, MI; 2) Albert Einstein College of Medicine, Bronx, NY; 3) University of Perugia, Perugia, Italy.

Toxoplasma gondii is a master manipulator capable of exploiting the resources from the host cell for its intracellular subsistence. However, the extent to which the parasite-host interactions influence the parasite’s nutrient acquisition remains to be elucidated. Residing within a non-fusogenic vacuole, the parasite encounters a barrier when it scavenges food that needs to cross the parasitophorous vacuole (PV) membrane. We discovered a potential role for the Endosomal Sorting Complex Required for Transport (ESCRT) machinery in host cytosolic protein uptake by T. gondii after disruption of host ESCRT function. Dense granule (GRA) proteins are important for remodeling and maintenance of the PV to support intracellular replication and for mediating host-microbe interactions. We have identified the transmembrane dense granule protein GRA14, that contains motifs homologous to the late domain motifs of the HIV Gag, as a candidate for the recruitment of the host ESCRT machinery to the PV membrane. Analysis of GRA14-deficient parasites revealed a marked reduction in ingestion of a host cytosolic protein compared to WT parasites. Using an HIV virus-like particle (VLP) release assay, we found that the motif-containing portion of GRA14 is sufficient for the recruitment of the host ESCRT machinery to the PV membrane. Further experiments demonstrated that GRA14-deficient parasites showed a reduced ability to activate STAT6, suggesting that GRA14 is required for the efficient activation of STAT6 in the parasite. Future studies are expected to provide insight into the role of this parasite in host cell activation, and how this interaction affects host cell metabolism and virulence.

32 The expression of a novel multigene family is correlated with channel activity in Babesia bovis-infected erythrocytes Hassan Hakim1, Thomas J Templeton2, Miako Sakaguchi3, Junya Yamagishi4, Shinya Miyazaki2, Kazuhide Yahata2, Shinichiro Kawazu1, Osamu Kaneko2, Masahito Asada1 1) National Research Center for Protozoan diseases (NRCPD), Obihiro University, Obihiro, Hokkaido, Japan; 2) Department of Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan; 3) Central Laboratory, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan; 4) Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan.

Babesia bovis, the most virulent causative agent of bovine babesiosis, extensively modifies the infected erythrocyte following invasion. This modification is done via the export of numerous proteins into the erythrocyte surface membrane and, to facilitate metabolite exchange, increase erythrocyte rigidity, and mediate cytoadherence in deep tissues. Despite their crucial role in virulence and pathogenesis such proteins, collectively called the “exportome,” have not been comprehensively characterized in B. bovis. We performed surface biotinylation of infected erythrocytes, followed by proteomic analysis. The approach was validated by the identification of a known surface exposed protein, variant erythrocyte surface antigen 1 (VES1A). Using immunofluorescence microscopy we confirmed the export of several candidates and found a novel exported protein with 10 transmembrane regions that is localized in spherical bodies and on the surface of infected erythrocytes. BLAST searches of PiroplasmaDB clarified that this protein is the product of a novel multigene family with 44 copies in the B. bovis genome, which we named mtm (multi-transmembrane protein). In the case of another erythrocyte-infecting apicomplexan parasite
Plasmodium falciparum}, blastocidin-S (BS) resistance was achieved by decreasing anion channel activity on the parasite-infected erythrocytes through epigenetic modification of one channel component's expression level. Thus, we generated BS-resistant \textit{B. bovis} to evaluate whether the expression of this protein is related to BS resistance. Development of BS resistance in \textit{B. bovis} resulted in downregulation of one major expressing \textit{mtm}, and overexpression of this \textit{mtm} in BS-resistant lines increased sensitivity to BS similar to the level of wild type parasites, supporting our hypothesis on the \textit{mtm} role in BS resistance. BS-resistant parasites-infected erythrocytes showed delayed lysis in sorbitol lysis assay confirming \textit{mtm} role in sorbitol uptake and possibly other solutes.

33 The esophageal gland mediates host immune evasion by the human parasite \textit{Schistosoma mansoni}. Jayhun Lee1,2, Tracy Chong1,2, Phillip Newmark1,2,3 1) Morgridge Institute for Research, Madison, WI; 2) Howard Hughes Medical Institute, University of Wisconsin–Madison, Madison, WI; 3) Department of Integrative Biology, University of Wisconsin–Madison, Madison, WI.

Schistosomes are parasitic flatworms that cause schistosomiasis, a major neglected tropical disease that affects over 200 million people globally. Their complex life cycle involves multiple body plans as they switch between sexual (in a molluscan host) and sexual (in a mammalian host) reproduction. As such, their successful propagation requires stem cells to undergo frequent transitions in their cycling and differentiation during parasite homeostasis and reproduction. As infectious larvae ( cercariae) arise from snails, a handful of stem cells packed inside the larval body serve as a likely origin for intra-mammalian parasitic development. However, how these early stem cells contribute to organogenesis remains unknown. Surprisingly, we found that the esophageal gland, an anterior accessory organ of the digestive tract, develops before the rest of the digestive system, and prior to blood feeding, suggesting that it may play a role in processes beyond nutrient uptake. To investigate the function of the esophageal gland, we characterized \textit{Sm-foxA} (\textit{foxA}), a gene encoding a forkhead-box transcription factor, that is highly enriched in the esophageal gland. Knockdown of \textit{foxA} completely blocked development and maintenance of the gland, without affecting other somatic tissues, as well as parasite viability, reproduction, and behavior in vitro. Intriguingly, schistosomes lacking the gland died after transplantation into naïve mice, while they were able to survive in immunodeficient mice lacking B-cells. Furthermore, feeding of GFP-expressing immune cells revealed that the gland-lacking parasites fail to lyse ingested immune cells within the esophagus before pasasing them into the gut. Together, this study unveils a novel immune evasion mechanism mediated by the esophageal gland, which is essential for parasite survival and pathogenesis.

34 Knockout of the UNC-51 like kinases ULK-4 and Fused disrupts motile flagella assembly in \textit{Leishmania mexicana}. Ciaran McCoy1, Humblende Vaucelle1, Tom Beneke1, Eva Gluenz1,2 1) Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; 2) Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK.

Homologues of ULK4 and Fused kinase have been linked to the function of cilia and flagella in diverse species. In \textit{Trypanosoma brucei}, they localise to the flagella connector (FC), a mobile transmembrane junction connecting the tip of the growing flagellum to the mature flagellum. In mice, which do not possess an FC, ULK4 and Fused kinase mutants display hydrocephalus, consistent with observed ciliogenesis defects in in distinct tissues and reduced ciliary motility. The mechanisms through which either protein contributes to motile cilia function are not well understood. We studied the phenotypes of \textit{ΔLmULK4} and \textit{ΔLmxFused} mutants in \textit{Leishmania}, which also lack the FC structure described for \textit{T. brucei}, and found that loss of either protein caused similar and severe defects in flagellar assembly: Only ~6% of the KO cells possess a flagellum that extends beyond the flagellar pocket (n=5000). KO mutants often exhibit atypical flagellar microtubule ultrastructure, with aberrant microtubule numbers and/or collapsed radial symmetry and reduced levels of the central pair-associated protein PF20. A subset of the KO cell populations that possess a long external flagellum displayed reduced numbers of both anterograde and retrograde intraflagellar transport particles relative to the parental control. Episomal addback of \textit{LmxFused} and \textit{LmxULK4} facilitated complete and partial rescue of the observed flagellar assembly phenotypes, respectively. Both proteins display a dispersed localisation throughout the cell body and flagellum, often with enrichment noted near the base and tip of the flagellum, as assessed via mNeonGreen tagging. This mNeonGreen signal was entirely detergent extractable, suggesting that these proteins are soluble. Interestingly, the stable expression \textit{LmxULK4}:mNG was largely dependent upon the presence of \textit{LmxFused}. A BioID screen with \textit{LmxFused::BirA*} provided evidence for close proximity of \textit{LmxULK4} and \textit{LmxFused}. Taken together these data suggest that \textit{LmxULK4} and \textit{LmxFused} may functionally interact in the same pathway to facilitate normal motile flagellum assembly.

35 A novel protein complex is essential for the maturation of transmission-stage malaria parasites Rebecca Clements1,2, Ershah Du2, Vincent Streva2, Jeffrey Dvorn1,2 1) Harvard Medical School; 2) Boston Children's Hospital.

Human malaria, which is caused by \textit{Plasmodium} parasites, remains an important cause of global morbidity and mortality. To successfully generate new antimalarials, we must gain a better understanding of the fundamental cell biology of \textit{Plasmodium falciparum}, the parasite responsible for the deadliest cases of malaria. A membranous scaffold and group of associated proteins called the inner membrane complex (IMC) serves as a structural support during major morphological changes throughout the life cycle of \textit{P. falciparum}, including segmentation of daughter cells during asexual replication and formation of transmission-stage parasites via gametocytogenesis. The basal complex lines the emerging edge of the IMC during segmentation and is likely critical for expansion of the IMC. It is unknown, however, what drives expansion of the IMC during gametocytogenesis. Here we describe the discovery of a novel basal complex protein, \textit{PfBLEB} (Baso-Lateral Expansion Boundary), PF3D7_0704300. Although \textit{PfBLEB} expression is not necessary for asexual replication \textit{in vitro}, we find that \textit{PfBLEB} is essential for gametocyte formation. Parasites lacking \textit{PfBLEB} harbor defects in IMC expansion and are unable to form mature, transmissible gametocytes. We demonstrate expression of \textit{PfBLEB} throughout gametocytogenesis, and find that \textit{PfBLEB} is part of a novel protein complex in gametocytes, which we name the gametocyte lateral complex. The gametocyte lateral complex is distinct in composition from the asexual basal complex, but similarly localizes to the expanding edge of the IMC. This study is the first demonstration of a role for a basal complex protein outside of asexual division, and, importantly, highlights a potential molecular target for ablation of malaria transmission.

36 Genome-wide screens identify \textit{Toxoplasma gondii} determinants of parasite fitness in IFNy-activated murine macrophages Yifan Wang1, Lamba Omar Sangaré1, Tatiana Paredes-Santos1, Musa Hassan2,3,4, Shruti Krishnamurthy1, Anna Furuta1, Benedict Markus5,6, SebastienLOURIDOS7, Jeroen SAEJ1 1) Department of Pathology, Microbiology & Immunology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA.; 2) College of Veterinary Medicine, The University of Edinburgh, Edinburgh, UK.; 3) The Roslin Institute, The University of Edinburgh, Edinburgh, UK.; 4) Center for Tropical Livestock Health and Genetics, The University of Edinburgh, Edinburgh, UK.; 5) Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA; 6) University of Freiburg, Faculty of Biology, Freiburg, Germany; 7) Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

\textit{Toxoplasma gondii} is an obligate intracellular parasite that infects the nucleated cell of almost all warm-blooded animals. Macrophages are the cell type preferentially infected by \textit{Toxoplasma in vivo} and play an essential role in the early immune response against the parasite. Interferon gamma (IFNy) elicits a variety of anti-Toxoplasma activities in macrophages. \textit{Toxoplasma} can block IFNy-induced parasite restriction mechanisms using effectors secreted from its unique secretory organelles. Many of these \textit{Toxoplasma} effectors determining parasite survival in IFNy-activated murine cells were initially identified using genetic crosses between strains that differ in virulence in mice. However, this approach fails to identify strain-independent genes. Here, using a genome-wide CRISPR screen we identified 353 \textit{Toxoplasma} genes that determine parasite fitness in naïve or IFNy-activated murine macrophages, seven of which we investigated and confirmed. We then focused on one of these genes encoding dense granule protein GRA45 because \textit{Ago45} parasites had significantly reduced virulence in mice and enhanced susceptibility to IFNy-mediated growth inhibition in macrophages from mice but also rats and humans. GRA45 has structural homology to bacterial small heat shock protein (sHSP) and has a conserved chaperone-like domain. Based on this, we discovered that GRA45 is critical for correct
localization of GRAs into the parasitophorous vacuole membrane and secretion of GRA effectors into the host cytosol. We further showed that the chaperone-like function of GRA45 plays an important role in maintaining IFNγ resistance in murine macrophages as parasite expressing GRA45 with either chaperone-like domain deletion or conserved sHSP motif mutations were more susceptible to IFNγ-induced growth inhibition. Together, we identified and characterized an important chaperone-like GRA in *Toxoplasma* and provided a resource for the community to further explore the function of *Toxoplasma* genes that determine fitness in IFNγ-activated macrophages.

37 Mitotic microtubules emanate from a novel intranuclear compartment of *Plasmodium falciparum* blood stage centrosomes Caroline S. Simon1, Ann-Kathrin Mehner1, Charlotta Funaya2, Yannik Voß1, Marta Machado1, Alexander Penning1, Severina Klaus1, Markus Ganter1, Julien Guizetti1 1) Centre for Infectious Diseases - Heidelberg University Hospital, Heidelberg, Germany; 2) Electron Microscopy Core Facility, Heidelberg, Germany.

Mitotic division in model organisms is typically followed by cytokinesis, leading to formation of two daughter cells. *Plasmodium falciparum*, however, divides by multiple asynchronous nuclear divisions not interrupted by cytokinesis. This leads to the formation of a multinucleated parasite stage called schizont. Although schizogony drives *P. falciparum* proliferation at several bottlenecks throughout its life cycle, the mechanisms underlying nuclear division are completely understudied. Centriolar plaques, the centrosomes of *P. falciparum*, are a likely key regulator of schizogony and serve as microtubule organizing centers. Aside from a few electron microscopy studies revealing an acentriolar structure, the detailed organization of centriolar plaques and mitotic microtubules is poorly understood.

Using STED nanoscopy, we describe three distinct nuclear tubulin configurations associated with schizogony, including tubulin foci, hemispindles and mitotic spindles. We attempt to reveal the chronology of these structures by live cell imaging of a Centrin1-GFP expressing cell line to mark the centrosome, in combination with live tubulin markers. Detailed analysis of centrin, nuclear pores and microtubule markers reveals a bipartite organization of the centriolar plaque. While centrin is clearly extranuclear, microtubules emanate from distinct nucleation sites within an intranuclear region. Despite being intranuclear this region, which is consistently associated with perinuclear centrin foci, is devoid of DNA. Correlative light microscopy and electron tomography analysis will reveal the nature of this novel DNA-free intranuclear compartment. This study enables us to build a working model of the ultrastructural organization of an unconventional centrosome to better understand the diversity of eukaryotic cell division modes.

38 Characterization of Trypanosoma cruzi Alpha tubulin acetyltransferase (ATAT) Victoria Alonso1,2, Mara Emilia Carloni2, Maria Cristina Motta4, Alejandro Pezza1, Maria Eugenia Chesta3, Gonzalo Martinez Peralta 1,2, Esteban Serra2,2 1) ICR-CONICET, Argentina; 2) Facultad de Ciencias Biológicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina; 3) Facultad de Medicina, Universidad Nacional de Rosario, Argentina; 4) Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.

The cytoskeleton of trypanosomatids has a simpler arrange than most eukaryotic cells. However, it is precisely organized and constituted by stable microtubules. Some microtubules compose the mitotic spindle, the basal body, the flagellar axoneme and the subpellicular microtubules, which are connected to each other and also to the plasma membrane, thus forming a helical arrangement along the central axis of the parasite cell body. Subpellicular, mitotic and axonal microtubules are extensively acetylated in *Trypanosoma cruzi*.

Acetylation on K40 of α-tubulin is conserved from lower eukaryotes to mammals and is associated with microtubule stability. It is also known that K40 acetylation occurs significantly on flagella, centrioles, cilia, basal body and the mitotic spindle. Several tubulin PTMs, including acetylation of K40, have been catalogued in Trypanosomatids, but the functional importance of these modifications for microtubule dynamics and parasite biology remains largely undefined. The primary tubulin acetyltransferase that delivers this modification was recently identified in several eukaryotes as Mec-17/ATAT, a Gcn5-related N-acetyltransferase. Despite evidence supporting a role for K40 acetylation in microtubule stability, its biological function in vivo is unclear.

We have expressed *T. cruzi* ATAT with an HA tag in epimastigotes using the inducible vector pTcINDEx-GW. Over-expressing parasites present a growth defect and also, we observed a diminished infectivity and an alteration in the differentiation from amastigotes to trypomastigotes. TcATAT is located in the cytoskeleton and flagella of *T. cruzi* as determined by western blot and confocal microscopy. Moreover, TcATAT colocalizes with acetylated alpha-tubulin in these structures and over-expression causes increased levels of the acetylated isoform and several morphological defects (cells with an abnormal DNA content and mitochondrial morphology). Also, over-expression causes a halt in the cell cycle progression of epimastigotes determined by flow cytometry and scanning electron microscopy. We observed that TcATAT acetylates α-tubulin in vitro and in vivo and is able to auto-acetylate. Finally, when ATAT is over-expressed we observed that parasites become more resistant to microtubule depolymerizing drugs.

These evidence supports the idea that tubulin acetylation is crucial for *T. cruzi* replication and differentiation and that TcATAT is responsible for this posttranslational modification.

39 Trypanosoma cruzi High Mobility Group B (TcHMGB): a pleiotropic player in trypanosome biology. Pamela Cribb1,2, Luis Tavernelli1, Virginia Perdomo2, Esteban Serra1,2 1) Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR; 2) Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina; 3) Facultad de Ciencias Medicas, Universidad Nacional de Rosario, Argentina; 4) Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.

High Mobility Group B (HMGB) proteins are conserved amongst eukaryotic factors involved in chromatin remodeling and important nuclear events like DNA transcription, replication, recombination and repair. Some HMGBs also play key roles outside the cell, acting as “alarmins” or Damage Associated Molecular Patterns (DAMPs) and are involved in the pathogenesis of many autoimmune, infectious and inflammatory chronic diseases.

TcHMGB, the HMGB from *Trypanosoma cruzi*, has two “HMG box” DNA-binding domains and a specific amino-terminal region unique of trypanosomatid HMGBs. This N-term sequence bears a nuclear localization signal (NLS) and a “DEK-C terminal” domain, which may confer special DNA-binding characteristics to the protein. Both the full length TcHMGB and its separated domains showed structural features such as the ability to bend linear DNA and bind to non-canonical structures like cruciform DNA in vitro. Also, TcHMGB can affect DNA structure in vivo, where its overexpression resulted in an altered chromatin state. Overexpression of TcHMGB also caused a dramatic decrease in epimastigotes growth possibly as a consequence of a delayed cell cycle progression with accumulation of parasites in G2/M phase and impaired cytokinesis. Amastigotes’ replication was also reduced in an in vitro infection model, as was the trypomastigotes’ infection efficiency and the number of finally released trypomastigotes. We propose that chromatin changes associated to the increased TcHMGB nuclear content, may affect parasite functions in sum resulting in a reduced fitness of the parasite.

Finally, we also demonstrated that TcHMGB, like human HMGB1, can be secreted out of the cell and induce the production of inflammatory mediators both in vitro and in vivo.

Taken together, our results suggest that the TcHMGB proteins can be considered a pleiotropic player involved not only in trypanosome key cellular processes, but also it should be considered a putative actor in Chagas disease pathogenesis.

40 A hurdle race to translate the coding sequence: translation in the presence of multiple upstream open reading frames happens via a mix of reinitiation and leaky scanning in *Plasmodium falciparum* Chhaminder Kaur1, Mayank Kumar2, Swati Patankar1 1) Indian Institute of Technology Bombay, Mumbai; 2) Amity Institute of Biotechnology, Amity University, Panvel.
A characteristic feature of *Plasmodium falciparum* is its AT-rich genome. Owing to this bias, transcripts in this parasite harbour an unusually high number of upstream open reading frames (uORFs). Similar to other eukaryotes, uORFs in *P. falciparum* pose a hurdle for ribosomes scanning for a start codon and thereby, repress translation of the main coding sequence (CDS). *P. falciparum* CDS have an average of 11 uORFs per transcript, and yet the cytoplasmic translation machinery of the parasite is able to carry out translation. This is possible due to a mix of reinitiation and leaky scanning and a systematic analysis of features such as Kozak sequence, length, codon composition and distance of the uORF from the CDS reveal that they play important roles in determining the extent of reinitiation and leaky scanning. Our study uncovers classes of genes that might be under translational regulation mediated via uORFs, thus adding another layer to the existing body of literature on gene regulation in *P. falciparum*. Among the major gene families harbouring repressive uORFs are var and ApalP2 transcription factor. We propose the existence of novel, parasite-specific proteins and/or modifications of existing translation factors to accommodate the extensive use of leaky scanning and reinitiation. Thus, in addition to the existing drug discovery efforts for organelar translation machinery, it appears that the cytosolic translation machinery also has the potential to contain novel targets for anti-malarial drugs.

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41 The interaction of Trypanosome Lytic Factor (TLF) with metacyclic promastigotes of *L. major* reduces C3b deposition and complement mediated lysis of the parasites*Jyi Pant1, Alan Sanchez1, Joseph Verdi3, Jayne Raper1,2 1) Hunter College, City University of New York; 2) The Graduate Center, City University of New York; 3) Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 28069120, Heidelberg, Germany.

Trypanosome Lytic Factor (TLF) is a high density lipoprotein (HDL) complex that represents 1% of total HDL in humans and higher order primates. Although originally discovered for its lytic activity against African Trypanosomes, we have shown that TLF ameliorates cutaneous infection by metacyclic promastigotes of cutaneous strains of *Leishmania* in mice and also in macrophages. Here, we studied the dynamic interaction of TLF with metacyclic promastigotes of *Leishmania* to understand the sequence of events before immune cells phagocytize the parasites. Using labelled TLF enriched HDL isolated from human plasma, we show that TLF binds to the metacyclic promastigotes of *Leishmania*. The binding of TLF to *Leishmania* metacyclic promastigotes does not kill/lyse the parasites. However, the binding of TLF to metacyclic promastigotes leads to a reduction in complement factor C3b deposition on the parasites compared to albumin controls. In order to include appropriate lipoproteins from transgenic mice producing TLF and compared it to lipoproteins from mice without TLF. Binding of TLF mice HDL to metacyclic promastigotes reduces the deposition of complement factor C3b compared to control mice HDL. This results in a reduction in complement mediated lysis of the metacyclic promastigotes. When phagocytosed by immune cells such as macrophages, bound TLF is activated by low pH and can kill the metacyclic promastigotes of *Leishmania*. Amastigotes of *Leishmania* however, do not bind TLF and are resistant to TLF lysis. We interpret these results as follows: TLF act as Trojan horse and binds to *Leishmania* metacyclic promastigotes leading to their protection from complement lysis. Thus TLF makes its way into immune cells together with the metacyclic promastigotes. Once within immune cells acidic phagosomes, TLF is activated and lyses the parasites leading to amelioration of infection.

42 The CLIP-domain serine protease CLIPC9 regulates melanization downstream of SPCLIP1, CLIPA8, and CLIPA28 in the malaria vector *Anopheles gambiae*Gregory L. Sousa1, Rikita Bishnoi2, Richard H. G. Baxter2, Michael Povelones1 1) University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; 2) Lewis Katz School of Medicine, Temple University, Philadelphia, PA.

The arthropod melanization immune response is activated by extracellular protease cascades predominantly comprised of CLIP-domain serine proteases (CLIP-Sps) and serine protease homologs (CLIP-SPHs). In the malaria vector, *Anopheles gambiae*, the CLIP-SPHs SPCLIP1, CLIPA8, and CLIPA28 form the core of a hierarchical cascade downstream of mosquito complement that is required for melanization. However, our understanding of the regulatory relationship of the CLIP-SPH cascade with the catalytic CLIP-Sps driving melanization is incomplete. Here, we report on the development of a novel screen to identify melanization pathway components based on the quantitation of infection-induced excreta, eliminating the need for microdissections or hemolymph enzymatic assays. Using this screen, we identified CLIPC9 and subsequent functional analyses established that this protease is essential for the melanization of both *Escherichia coli* and the rodent malaria parasite *Plasmodium berghei*. Mechanically, septic infection with *E. coli* promotes CLIPC9 cleavage and both full-length and cleaved CLIPC9 localize to this bacterium in a CLIPA8-dependent manner. The steady state level of CLIPC9 in the hemolymph is regulated by thioester-containing protein 1 (TEP1), suggesting it functions downstream of mosquito complement. In support, CLIPC9 cleavage is inhibited following SPCLIP1, CLIPA8, and CLIPA28 knockdown positioning it downstream of the CLIP-SPH cascade. Moreover, like CLIPA8 and CLIPA28, CLIPC9 processing is negatively regulated by serine protease inhibitor 2 (SRPN2). This report demonstrates how our novel excretion-based approach can be utilized to dissect the complex protease networks regulating mosquito melanization. Collectively, our findings establish that CLIPC9 is required for *An. gambiae* melanization and shed light on how the CLIP-SPH cascade regulates this potent immune response.

43 Characterization of Trypanosomes from Vectors, Skin and Blood of Cattle in Southern GhanaWilliam Eklohi1,2, Jack Sunter3, Jonathan P. Adjimani1,2, Yahaya Adams1, Benita Djamah5, Keith Gul4, Theresa Manful Gwira1,2 1) West African Centre for Cell Biology of Infectious Pathogens, University of Ghana; 2) Department of Biochemistry, Cell and Molecular Sciences, Oxford Brookes University, UK; 3) Department of Biological and Medical Sciences, Oxford Brookes University, UK; 4) Sir William Dunn School of Pathology, Oxford University, UK; 5) Tssetse and Trypanosomiases Control Unit of Veterinary Services Department, Ghana.

Animal African trypanosomiasis (AAT) a major burden to livestock production is transmitted by tsetse flies and other biting flies. The parasite has historically been described as blood-borne but recent researches have shown the parasites sequestering in adipose tissues and the skin. This study aims to identify and characterize circulating strains of trypanosomes in the vectors, skin and blood of cattle before and after treatment with diminazene aceturate in a high endemic area. The number and species of the vectors were determined using biconical traps in March and July, 2018. Molecular identification of trypanosomes in the insect vectors, skin biopsies and blood of cattle was done by a nested multiplex PCR based on the alpha-beta tubulin gene. A total of 2801 flies were trapped and identified as 2 *Glossina tachinoides*, 24 *Tabanid kingi*, 145 *Stomoxys calcitrans*, 2586 others and 44 *Musca domestica*. The average fly per day was higher during the July sampling compared to March and the highest number of flies were identified in the kraal. Mechanical transmitters tabanids and stomoxys were the main vectors of AAT at the study site. Molecular identification showed that *T. brucei brucei*, *T. congolense*, *T. vivax* and some mixed infections were detected in the vectors, skin biopsies and blood of the cattle. The most abundant species detected was *T. brucei brucei*. It was also shown that trypanosomes were able to sequester under the skin from a natural infection as well as the blood of the cattle before and after treatment with diminazene aceturate.

44 Common genetic variation in human red blood cells drives growth rate variation in *P. falciparum* parasitesEmily R Ebell1, Frans A Kuyers2, Carrie Lin1, Dmitri A Petrov1, Elizabeth S Egan1 1) Stanford University, Stanford, CA; 2) Children's Hospital Oakland Research Institute, Oakland, CA.

Malaria caused by the replication of *Plasmodium falciparum* parasites within red blood cells (RBCs) has killed hundreds of millions of people, especially in Africa. Human RBCs harbor considerable genetic variation that, if important for parasite fitness, could be subject to positive selection from malaria. However, aside from a handful of pathogenic alleles, the effects of RBC variation on *P. falciparum* replication is poorly understood. To address this gap, we performed exome sequencing, *P. falciparum* growth and invasion assays, and extensive RBC phenotyping on blood samples from 122 donors, mostly healthy individuals with African ancestry. Sequencing revealed that 40% of donors with no reported history of blood disorders carried alleles for G6PD deficiency, α-thalassemia, HBAs, or HbAC. Both carriers and non-carriers displayed wide, overlapping ranges of RBC phenotypes and susceptibilities to *P. falciparum*. By applying LASSO regression, we found that non-carrier phenotypes including RBC size, deformability, and hydration status explained up to 40% of the observed
variation in parasite growth. With the addition of genetic variation from 23 malaria-related proteins, our model explained up to 83% of the observed variation in parasite growth among non-carriers. SPTA1, SPTB, and other components of the flexible membrane backbone contained several polymorphisms associated with Plasmodium fitness, as did the ion channel PIEZO1. Unexpectedly, we found that RBCs with more African ancestry were not enriched for protective polymorphisms and were less likely to display phenotypes that inhibited Plasmodium. These findings suggest a model in which Plasmodium fitness in healthy red blood cells is controlled by the combined effects of common variation in a moderate number of host genes. Unraveling the pleiotropic mechanisms through which this common genetic variation impacts red cell function and malaria resistance may lead to new therapies for this ancient disease.

45 The key to egress? Babesia bovis perforin-like protein 1 (PLP1) with hemolytic capacity is required for blood stage replication and is involved in the exit of the parasite from the host cell. Martina Paoletta1, Jose Jaramillo Ortiz1, Ludmila Lopez Arias1, Paul Lacy2, Jacob M. Laugher3, Carlos Sauer2,3, Marisa Farber1, Silvina Willkowsky1 1 Instituto de Agrobiotecnología y Biología Molecular (IABIMO) - INTA-CONICET, Argentina; 2) Animal Disease Research Unit, USDA-ARS, Washington State University, 3003 ADBF, Pullman, WA, USA; 3) Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.

Bovine babesiosis is a tick-borne disease caused by Babesia parasites affecting livestock production worldwide. Perforin-like proteins (PLP) are apicomplexan proteins with the capacity of generating pores in lipid bilayers and, although they have been described as key factors in host-pathogen interaction in related pathogens, their role in Babesia remains unknown. We have previously identified in B. bovis a family of six PLPs that might be involved in pore formation and red blood cell (RBC) damage, and demonstrated that one member, PLP1, is expressed and exposed to the host immune system during infection. The aim of this study was to determine the function of PLP1 and its contribution to parasite’s pathogenesis.

The recombinant MACPF domain (responsible for pore formation in PLPs) of PLP1 was expressed and hemolysis assays were done incubating this protein with bovine RBCs. Cell lysis was expressed as a percentage of maximum hemoglobin release with Triton X-100 treatment. High hemolysis levels (> 80%) were obtained at [MACHP] > 80 nM, and pH > 5, which supports the pore forming function of the protein. The hemolysis activity was not affected by changes in [Ca2+], and a dose-response curve reflected a cooperative-positive response of the protein.

A B. bovis knock out (KO) strain was generated by disruption of plp1. Parasites were transfected with a plasmid to guide replacement of plp1 with an egfp-bsd fusion gene that acts as a reporter and a selectable marker. The efficient replacement of plp1 was confirmed by PCR and sequencing, and a clonal line was generated by FACS assays. The in vitro replication of the KO in bovine RBC cultures was evaluated. Results showed a decreased growth rate compared to the wild type strain and a peculiar phenotype consisting of multiple parasites within a single RBC suggesting that the lack of PLP1 has a negative impact on the parasite’s egress and in its capacity to proliferate.

We conclude that PLP1 is a pore forming protein involved in the egress of the parasite from the host cell and, even though it is not essential, plays an important role in B. bovis blood stages. Further studies will be focused on determining if the replication defect results in an attenuated phenotype in vivo and the potential use of this strain as a genetically attenuated vaccine.

46 Phospholipase activity within the parasitophorous vacuole is essential for blood stage growth and efficient egress in Plasmodium falciparum Abhinay Ramprasad1, Paul-Christian Burda2,3,4, Konstantinos Kousis1, James A Thomas1,5, Enrica Calvani1, Chrissiane Withers-Martinez1, Christine R Collins1, James MacRae1, Tim-Wolf Gilberger2,3,4, Michael J Blackman4,5 1) The Francis Crick Institute; 2) Centre for Structural Systems Biology; 3) Bernhard Nocht Institute for Tropical Medicine; 4) University of Hamburg; 5) London School of Hygiene & Tropical Medicine.

Malaria parasite egress from host red blood cells involves parasite-mediated membrane rupture in which membrane-lytic effector molecules such as perforin-like proteins and phospholipases have been proposed to be involved. Here we have used a combination of conditional gene disruption, proteomics and lipidomics strategies to identify these effector molecules in Plasmodium falciparum. We show that perforin-like proteins have no important role in asexual blood stage egress. Instead, a phospholipase (PF3D7_0629300) expressed in the parasitophorous vacuole (PV) plays a role in membrane remodelling that allows efficient parasite egress. Moreover, we report significant depletion of an unusual phospholipid species, acylphosphatidylglycerol, during egress. We also show that a PV-resident glycerophosphodiesterase (PF3D7_1406300) is essential for parasite growth during trophozoite stages and could be involved in acquiring choline from host lysophosphatidylcholine. We conclude that phospholipid breakdown and remodelling within the PV lumen is critical for asexual growth and to facilitate egress in malaria parasites.

47 Babesia divergens cGMP-dependent kinase (PKG) is required to exit the replication cycle and initiate egress Brendon Elsworth1, Caroline Keracki1, Yasaman Rezvani2, Jacob Tennessen1, Samantha Sack1, Aditya Paul1, Cristina Moreira1, Jonathan Goldberg1, Kourosh Zarringhalam2, Manoj Duraisingh1 1) Harvard T. H. Chan School of Public Health, Boston, MA.; 2) University of Massachusetts - Boston, Boston, MA.

A unique aspect of apicomplexan parasite biology is the requirement of egress from and invade their host cells. Babesia spp. parasites proliferate in and eventually destroy the red blood cells (RBC) of their mammalian host, leading to severe and sometimes fatal illness. Many of the mechanisms, and the molecular mediators, of parasite egress have been characterized in Plasmodium spp. and T. gondii, with conserved and divergent features. Egress remains poorly characterized in other apicomplexan parasites, including Babesia spp. Through the use of small molecule inhibitors, we have implicated cGMP/PKG signaling, intracellular and extracellular calcium, proteases, and gliding motility in the egress of B. divergens. Parasites can be efficiently induced to egress with the cGMP analog, 8-Br-cGMP, throughout the majority of the replication cycle, but most efficiently in mature parasites. To further identify the molecular mediators of egress, we used a transcriptomic approach. A synchronous transcriptome of the B. divergens replication cycle was generated, which revealed co-regulation of proteins expected to localize to the same subcellular compartment by predicted orthology with T. gondii and P. falciparum. Analysis of co-regulated genes identified putative effector molecules of egress and invasion that have not previously been studied in Babesia spp., informing a model framework for regulating egress. To further characterize egress, we developed and employed reverse genetic and chemical genetic tools to target B. divergens cGMP-dependent kinase (PKG), a well characterized mediator of egress in Plasmodium spp. and T. gondii. B. divergens PKG is essential for parasite growth and is a druggable target that is absolutely required for parasite egress as well as invasion. Strikingly, in the absence of PKG function, non-egressed parasites continue for several more rounds of replication intracellularly, indicating that the kinase is required for exit from the replication cycle. All taken together, despite the closer evolutionary distance and a shared host cell niche with Plasmodium spp., the egress process of Babesia divergens more closely resembles that of the more distantly related Toxoplasma gondii.

48 Actomyosin forces and the energetics of red blood cell invasion by the malaria parasite Plasmodium falciparum Thomas Blake1, Silvia Haase1, Jake Baun1 1) Imperial College London.

Red blood cell (RBC) invasion by Plasmodium spp. merozoites is at the heart of malaria pathogenesis. Merozoite invasion has been systematically studied by video microscopy for 50 years and its distinct and ordered phases have been well established for much longer. Merozoite entry is rapid, completed within 30 seconds of RBC contact, and active, powered by a parasite actomyosin motor. The canonical model for merozoite force production has arrays of a myosin motor (myosin A, MyoA) working against short actin filaments connected via transmembrane adhesins to the substrate. We recently showed that MyoA
from the most virulent malaria parasite, *Plasmodium falciparum*, PfMyoA, is critical for merozoite invasion (PMID: 31337750). However, work on other parasites has pointed to retrograde flow of parasite plasma membrane and host cell activity as other sources of force production, meaning that the precise function of PfMyoA in invasion, its regulation, the role of other myosins and overall energetics of invasion remain unclear.

To dissect the roles of PfMyoA, its essential light chain PfELC and an auxiliary motor PfMyoB during merozoite invasion, we developed a conditional mutagenesis strategy and captured almost 700 invasion events by time-lapse video microscopy. By imaging mutants with increasing defects in force production, based on disruption to a parasite-specific phospho-regulation site, the absence of PfELC or complete absence of the PfMyoA motor, we define three distinct stages of incomplete RBC invasion. These three defects reveal three energetic barriers to successful entry: RBC deformation (pre-entry), mid-invasion initiation of entry, and completion of internalisation, each requiring an active parasite motor. In defining distinct energetic barriers to invasion, these data illuminate the mechanical challenges faced in this remarkable process of protozoan parasitism, highlighting distinct myosin functions and identifying potential targets for preventing malaria pathogenesis.

49 Divergent proteins of unknown function in a *Plasmodium mitoribosomal complex* 

Swati Dass1, Maruthi Mulakal1, Michael Mather1, Akhil Vaidya1, Hangjun Ke1 1) Drexel University.

Mitochondrial ribosomes (mitoribosomes) are the evolutionary descendent of bacterial ribosomes. In many eukaryotes, these complexes specialize in co-translational insertion of membrane proteins that are critical for mitochondrial functions. Mitoribosomes have a conserved composition of core ribosomal RNAs (rRNAs) and ribosomal proteins (RPs), where rRNA plays the enzymatic role of protein translation. Their RPs have undergone many species-specific variations across eukaryotic lineages, which include expansion in the size of conserved ribosomal proteins and recruitment of many additional novel and divergent proteins. In parasitic protozoa, the overall trend shows an increase in protein content that balances a reduction of rRNA. The recently reported structure of *Trypanosoma brucei* mitoribosome provides an example in which the rRNA content is highly reduced (SSU: 620 nt, LSU: 1176 nt), and the protein content has risen to 127 polypeptides, most of which are unique to Kinetoplastids. In *Plasmodium spp.*, mitochondrial rRNA is not only reduced, but are also highly fragmented into 20-200 nt pieces; while, *Plasmodium* putatively encodes 14 small subunit (SSU) and 30 large subunit (LSU) RPs. We have reported that knocking down SSU (S12, S17) [PMID: 32273345] and LSU (L13) RPs [PMID: 29626906] is lethal to the parasites. Further, we have generated 7 endogenously tagged lines by adding 3HA to SSU (S12, S15, S17, S18, S22) and 2 LSU (L13, L23) RPs. Interestingly, all these RPs are detected in a native complex of about 0.8 MDa, suggesting that they belong to a common complex. Preliminary mass spectrometry data from one parasite line (expressing L23_3HA) suggest that several unknown proteins associated with this complex may be a part of the mitochondrial translational machinery. Using these parasite lines, we intend to pull down the complex and find the missing players of the *Plasmodium* mitoribosome.

50 Understanding the role of mitochondrial-pellicle membrane contact sites in *Toxoplasma gondii* 

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*Toxoplasma*’s singular mitochondrial is extremely dynamic and undergoes morphological changes throughout the parasite’s life cycle. While intracellular, the mitochondrion is maintained in a lasso shape that stretches around the parasite periphery and is in close proximity to the pellicle, suggesting the presence of membrane contact sites. Upon egress, these contact sites disappear, and the mitochondrion retracts and collapses towards the apical end of the parasite. Once reinvaded, the lasso shape is quickly reformed, indicating that dynamic membrane contact sites regulate the positioning of the mitochondrion. We discovered a novel protein (TgGT1_265180) that associates with the mitochondrion via interactions with the fission related protein Fis1. Knockout of TgGT1_265180, which we have dubbed LMF1 for Lasso Maintenance Factor 1, results in a complete disruption of the normal mitochondrial morphology. In intracellular LMF1 knockout parasites, the mitochondrion lasso shape is disrupted, and instead it is collapsed as normally only seen in extracellular parasites. Additionally, proper mitochondrial segregation is disrupted, resulting in parasites with no mitochondrial and extra mitochondrial material outside of the parasites. These gross morphological changes are associated with a significant reduction of parasite propagation and can be rescued by reintroduction of a wildtype copy of LMF1. Co-immunoprecipitations and Yeast Two-Hybrid predict interactions with the parasite pellicle. Therefore, we hypothesize that LMF1 mediates contact between the mitochondrion and the pellicle in a regulatable fashion, and that the LMF1-dependent morphodynamics are critical for parasite propagation. Current studies are focused on characterizing the consequences of mitochondrial collapse and identifying proteins that interact with LMF1 to position the mitochondrion to the periphery of the parasite.

51 Developmental regulation of edited Cyb and COII mitochondrial mRNAs is achieved by distinct mechanisms in *Trypanosoma brucei* 

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*Trypanosoma brucei* is a parasitic protozoan that undergoes a complex life cycle involving insect and mammalian hosts that present dramatically different nutritional environments. Mitochondrial metabolism and gene expression are highly regulated to accommodate these environmental changes, including regulation of mRNAs that require extensive uridine insertion/deletion (U-indel) editing for their maturation. Here, we use high throughput sequencing and a method for promoting life cycle changes in vitro to assess the mechanisms and timing of developmentally regulated edited mRNA expression. We show that edited Cyb mRNA is downregulated in mammalian bloodstream forms (BSF) at the level of editing initiation and/or edited mRNA stability. In contrast, edited COII mRNA is upregulated in mammalian BSF at the level of editing initiation and/or edited mRNA stability. We find that the timing of edited mRNA levels is not correlated with the timing of parasite life cycle stages, suggesting that edited mRNA levels are regulated by additional mechanisms.

The current studies are focused on characterizing the consequences of mitochondrial collapse and identifying proteins that interact with LMF1 to position the mitochondrion to the periphery of the parasite.

52 Site-Specific and Substrate-Specific Control of Accurate mRNA Editing by a Helicase Complex in *Trypanosomes* 

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Trypanosoma U-insertion/deletion RNA editing in mitochondrial mRNA involves thousands of enzymatic reactions directed by small antisense transcripts termed guide RNAs (gRNAs). Editsomes include the RNA Editing Catalytic Complex (RECC) and the auxiliary RNA Editing Substrate binding Complex (RESC) and RNA Editing Helicase 2 Complex (REH2C). The process is highly “error-prone” since most steady-state mRNAs are partially edited and include mis-edited ‘junction’ regions that match neither pre-mRNA nor fully-edited transcripts. Understanding the molecular mechanisms that determine the balance between “incorrect” and “correct” edits (i.e., editing accuracy) remains paramount. In the editing model, RESC serves as a platform for productive mRNA–RECC enzyme contacts. Protein subunits of REH2C promote accurate editing on RESC. However, the functional interplay between RESC and REH2C remains unclear.
How does REH2C affect editing? Does REH2C target specific RNA sites. Does REH2C affect all mRNAs equally? To address these questions, we applied a novel nucleotide-resolution RNA-seq approach to examine hundreds of positions, one-by-one, in two mRNAs encoding: Ribosomal Protein Subunit 12 (RPS12) and ATPase-subunit 6 (A6). We directly compared transcripts associated with RESC and REH2C to those found in total mitochondrial RNA. RESC-associated transcripts exhibited site-preferential enrichments in total and accurate edits. REH2C loss-of-function induced similar substrate-specific and site-specific editing effects in RESC and in total mtrNA. Surprisingly, REH2C loss decreased total editing primarily at RPS12 5' positions but increased total editing at examined A6 3' positions. REH2C loss caused a site-preferential decrease of accurate editing in both transcripts. However, changes in total or accurate edits did not necessarily involve common sites. Our studies also revealed important features of early editing in in RPS12 and A6 mRNAs. A few 5’ nucleotides of the initiating gRNA (gRNA-1) directed accurate editing in both transcripts. However, in RPS12, two conserved 3’-terminal adenines in gRNA-1 could direct a non-canonical 2U-insertion that causes major pausing in 3’-5’ progression. In A6, a non-canonical sequence element that depends on REH2C in a region normally targeted by the 3’-half of gRNA-1 may hinder early editing progression. Overall, we defined transcript-specific effects of REH2C loss in general, and at least in A6, REH2C-mediated effects in early editing.

53 Complexome profile of Toxoplasma gondii mitochondria identifies a divergent cytochrome bc1 complex Andrew Maclean1, Hannah Bridges2, Mariana Silva3, Judy Hirst2, Ulla Sheiner3 1) Wellcome Centre for Integrative Parasitology, University of Glasgow, U.K.; 2) MRC Mitochondrial Biology Unit, University of Cambridge, U.K.; 3) Institute of Biomedical Sciences, Federal University of Uberlândia, Brazil.

The mitochondrial electron transport chain (mETC and F1F0-ATP synthase are of central importance for energy and metabolism in eukaryotic cells. The Apicomplexa, important pathogens of humans causing diseases such as toxoplasmosis and malaria, depend on their mETC in every known stage of their complex life cycles. Here, using a complexome profiling proteomic approach, we have characterised the chain of Toxoplasma mETC complexes and F1F0-ATP synthase. We identified and assigned 60 proteins to complexes II, IV and F1F0-ATP synthase of Toxoplasma, of which 16 are novel. Furthermore, our complexome profile elucidates the composition of the Toxoplasma complex III, the target of clinically used drugs such as atovaquone. We identified two new homologs and two novel-parasite-specific subunits, one of which are broadly conserved in myzozoans. We demonstrate all four proteins are essential for parasite growth and for complex III formation, and show that their depletion leads to a defect in mitochondrial potential, supporting their role as complex III subunits. Our study highlights the divergent subunit composition of the mETC complexes in apicomplexans and sets the stage for future structural and drug discovery studies.

54 Deciphering the mitochondrial quality control mechanism in the parasite Trypanosoma brucei Caroline Dewari1, Silke Oeljeklaus1, Ida Suppan2, Christian Heikert, Jan Mani1, Bettina Warscheid1, André Schneider1 1) DCB, University of Bern, Switzerland; 2) Institute of Biology II, University of Freiburg, Germany.

Mitochondrial quality control (MQC) is the network of pathways by which eukaryotic cells monitor and maintain the function of their mitochondria. We study this process in the protist Trypanosoma brucei. This organism has a large single mitochondrion, which prevents the elimination of individual dysfunctional mitochondria as in some other organisms. Interestingly, we have found orthologs of most common MQC components are absent in T. brucei. More and more examples of convergent evolution are being discovered in T. brucei, particularly with regards to mitochondrial biogenesis, and we expect the same to be the case for mechanisms governing MQC.

We will show data demonstrating the existence of a MQC pathway in T. brucei which is triggered when mitochondrial protein import is blocked, where the endpoint is the proteasomal degradation specifically of mislocalised mitochondrial proteins. We show that the proteasome and ribosome are recruited to the mitochondrion upon the induction of an import defect, along with several trypanosomatid-specific proteins which we have now shown are required for this MQC pathway. These candidates are now being investigated as to their roles within a putative ubiquitination cascade. Of particular interest is a nuclearly-localised protein with a ubiquitin-like domain, which appears to be involved in the cytoplasma upon the induction of a mitochondrial protein import defect. This process seems to be required for the MQC mechanism to act.

Evidence supporting the presence of a second MQC pathway will also be presented, which extracts and degrades destabilised membrane proteins from the outer mitochondrial membrane. Curiously, although homologs of known AAA-ATPase proteins MSP1 and p97/ecd48 seem to play roles in this pathway, they appear to be utilising noncanonical cofactors.

55 Mitochondrial keto-acid dehydrogenases are essential for the generation of acetyl-CoA in asexual Plasmodium falciparum parasites Justin Munro1,2, Sethu Nair3, Sean Prigge3, Manuel Ulñias1,2,4 1) Department of Chemistry, Pennsylvania State University; 2) Huck Center for Malaria Research, Pennsylvania State University; 3) Bloomberg School of Public Health, Johns Hopkins University; 4) Biochemistry and Molecular Biology Department, Pennsylvania State University.

Coenzyme A (CoA) biosynthesis has recently been demonstrated to be an excellent target for antimalarial intervention. While most studies have focused on the predominant modifications of CoA into acetyl-CoA in the apicoplast and the cytosol, mitochondrial acetyl-CoA production is less well understood. The current model for acetyl-CoA generation comes from experiments conducted in Plasmodium berghei parasites demonstrating that the branched chain keto-acid dehydrogenase (BCKDH) enzyme acts as a mitochondrial pyruvate dehydrogenase and is crucial for the generation of acetyl-CoA and for normal development within erythrocytes. Mitochondrial keto-acid dehydrogenase enzymes, such as BCKDH, are dependent upon lipoylation driven by the lipoylase ligase (LipL) 2 enzyme. In this study, we set out to investigate the role of BCKDH and LipL2 on the generation of acetyl-CoA and asexual growth of P. falciparum by using a combination of gene knockouts followed by growth and metabolomics assays. We find that P. falciparum parasites bckdh knockout lines grow normally showing that BCKDH alone is not crucial for parasite development. However, a lipL2 knockout line resulted in a complete growth arrest. This arrest could be rescued by supplementation with millimolar amounts of acetate (5 mM). Similarly, a double knockout line of both the bckdh and the alpha-ketoglutarate dehydrogenase (kdh) e2 subunits, grows only when supplemented with acetate. We carried out 13C-glucose and 13C-glutamine, and 13C-acetate metabolite labelling experiments to trace the metabolic contribution of these precursor molecules during acetate supplementation in the lipL2 knockout. As expected, in the absence of 5 mM acetate, acetate was readily incorporated into acetyl-CoA to the point where acetyl-CoA from glucose was nearly undetectable in both the lipL2 KO line and the wild-type control. After establishing that the acetate primarily boosted acetyl-CoA generation, we investigated the metabolism of a lipL2 knockout line, a bckdh e2 and kdh e2 double knockout line, and a bckdh e1 and e2 double knockout line using 13C-glucose and 13C-glutamine labelling in the absence of exogenous acetate. In all of the tested knockout lines, we found that acetyl-CoA generation was severely depleted relative to the wild-type line. These results confirm that BCKDH is indeed acting as a mitochondrial pyruvate dehydrogenase. Further, our results suggest that KDH performs a function that rescues the metabolic defect of a bckdh KO, although KDH is not capable of generating a notable increase in acetyl-CoA. Taken together, our results demonstrate that P. falciparum critically relies on the mitochondrial generation of acetyl-CoA, an essential process which may be targeted with great success to kill asexual P. falciparum parasites.

56 Plastid biogenesis in malaria parasites requires the interactions and catalytic activity of the Clp proteolytic system Anat Florentin1,2, Dylon Stephens1, Carrie Brooks1, Vasant Muralidharan1 1) Hebrew University in Jerusalem; 2) University of Georgia.

The human malaria parasite, Plasmodium falciparum, contains an essential plastid called the apicoplast. Most apicoplast proteins are encoded by the nuclear genome and it is unclear how the plastid proteome is regulated. Here, we study an apicoplast-localized caspase-like protease (Clp) system and how it regulates organelle proteostasis. Using null and conditional mutants, we demonstrated that the Clp protease (PfClpP) has robust enzymatic activity that is essential for apicoplast biogenesis. We developed a CRISPR/Cas9 based system to express catalytically-dead PfClpP, which showed that PfClpP oligomerizes as a
zymogen and is matured via trans-autocatalysis. The expression of both wild type and mutant Clp chaperone (PfClpC) variants revealed a functional chaperone-protease interaction. Conditional mutants of the substrate-adaptor (PfClpS) demonstrated its essential function in plastid biogenesis. A combination of multiple affinity purification screens identified the Clp complex composition as well as putative Clp substrates. This comprehensive study reveals the molecular composition and interactions influencing the proteolytic complex of the apicoplast Clp system and demonstrates its central role in the biogenesis of the plastid in malaria parasites.

57 An Alveolata secretory machinery adapted to parasite-host cell invasion Eleonora Aquilini1, Marta Cova1, Diana Penarete-Vargas1, Rania Najm1, Arnaud Graindorge1, Daniela Sparvoli1,2, Nicola Pacheco1, Catherine Suare1, Marjorie Maynadier1, Laurence Berry-Sterkers1, Serge Urbach3, Pilar Fahy4, Jean-François Dubremetz1, Aaron Turkewitz2, Maryse Lebrun3 1) UMR 5235 CNRS, University of Montpellier, France; 2) Department of Molecular Genetics and Cell Biology, University of Chicago, USA; 3) IGF CNRS, Université de Montpellier, France; 4) Pôle Facultaire de Microscopie Ultrastructurale, Geneva, Switzerland.

Apicomplexa possess specialized secretory organelles that undergo regulated exocytosis during invasion. Rhoxy proteins are injected directly into the host cell to support invasion, vacuole formation, and subversion of host immune function. The mechanism by which they are discharged is unclear, but appears distinct from those used by bacteria, yeast, animals or plants. Together with ciliates and dinoflagellates, Apicomplexa parasites belong to the Alveolata superphylum. Previous studies in the ciliate Paramecium tetraurelia showed that upon stimulation their defensive organelles fused with a "rosette" of 8–10 particles embedded in the plasma membrane, whose formation is dependent on Nd proteins "Nd for Non Discharged". Remarkably, a similar structure was observed at the apex of several apicomplexan parasites, which still remains uncharacterized.

Here we have identified and characterized the orthologs of nd6 and nd9 in the apicomplexan model Toxoplasma gondii. Depletion of Tgd6 and Tgd9, abolished rhoxy secretion and blocked host-cell invasion and parasite development. We next demonstrated that Plasmodium falciparum Nd9 is also required for rhoxy exocytosis. We also showed that T. gondii Nd9 mutants lacked the apical rosette, thereby establishing a link between rosette and rhoxy exocytosis. To shed more light on our understanding of the molecular composition of the rhoxy secretion machine, we searched for Nd9 interacting proteins in T. gondii. This analysis revealed robust interaction of Nd9 with Nd6 and with TgFER2, a member of the ferlin calcium sensor family, known to be essential for Toxoplasma rohytpy secretion. This complex includes also proteins unique to Alveolata that we experimentally linked to rhoxy secretion in Toxoplasma and muncyos secretion in the ciliate Tetrahymena. These proteins and their domains yield a regulatory model in which calcium signaling and nucleotide binding and hydrolysis constitute key steps that control rosette function and organelle discharge. The rosette and the set of Nd proteins highlight a common ancestry for the fusion machinery linked to secretory organelles in groups of protists that diverged hundreds of millions of years ago and have adopted radically different lifestyles.

58 Rapid Biodi in Plasmodium ookinetes identifies a protein with dual function during mosquito infection Jessica Kehrer1, Dominik Rickens1, Leanne Straub1, Emma Pietsch1, Julia Heinze1, Friedrich Frischknecht1 1) University of Heidelberg Medical School, Integrative Parasitology.

Transmission of the malaria parasite Plasmodium to mosquitoes necessitates gamete egress from red blood cells to allow zygote formation and ookinete motility, needed to penetrate the midgut epithelium. Both processes are dependent on the secretion of proteins from different sets of specialized vesicles. Inhibiting some of these proteins, has shown potential for blocking parasite transmission to the mosquito. In order to identify new transmission-blocking vaccine candidates, we defined the microneme content of Plasmodium berghei ookinetes using Apex2-mediated rapid proximity-dependent biotinylation. This identified novel micronemal proteins along with over 50 novel candidates and sharpened the list of a previous survey, based on subcellular fractionation. Functional analysis of the most promising candidate, containing a signal peptide and 4 transmembrane domains uncovered a dual function in male gametogenesis and ookinete midgut traversal. Strikingly, mutation of a putative trafficking motif in the C-terminus affected ookinete to oocyst transition but not gamete formation. This suggests the existence of distinct transport requirements in different parasite stages.

59 Loss of a conserved MAPK causes catastrophic failure in assembly of a specialized cilium-like structure in Toxoplasma gondii William O'Shaughnessy1, Peter Back2, Pravin Dewangan1, Kai Cai1, Peter Bradley2, Daniela Nicastro1, Michael Reese1 1) UT Southwestern Medical Center, Dallas, TX; 2) Molecular Biology Institute, University of California, Los Angeles, CA.

Apicomplexa are named for the complex of secretory organelles and invasion machinery organized at the parasites apical tip (the "apical complex"). Using Toxoplasmosa as a model, we found that the MAPK ERK7 is essential for correct biogenesis of the apical complex. Without active ERK7, parasites fail to develop mature ciliates, the organizing core of the Toxoplasma apical complex. Furthermore, we found that the apical cap protein, AC9, acts as a regulatory scaffold that controls ERK7 localization and activity. These discoveries provide a foundation to begin to dissect the regulation of apical complex biogenesis and function. CryoET (electron tomography) and cryoFIB (focused-ion-beam) allow the elucidation of large macromolecular complexes in their native states in flash-frozen cells with intact membranes. We will share recent data harnessing this technology to define the structures of the apical complex actively engaged in secretion.

60 Screening the TCAMS library of small molecules for inhibitors of AMA1-RON2 interaction Geervani Daggupati1, Adam Yasgar2, Maria Jesús Almela-Armendáriz3, Maria Isabel Castellote-Alvaro3, Elena Fernandez Alvaro3, Francisco Javier Gamo3, Anton Simeonov2, Dolores Jimenez-Alfaro-Mtnez 3, Louis Miller4, Praakash Srinivasan1 1) 1Malaria Research Institute, Dept. Molecular Microbiology and Immunology, Johns Hopkins School of Public Health, Baltimore, MD 21205; 2) National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20850; 3) Tres Cantos Medicine Development Campus, GlaxoSmithKline, Parque Tecnológico de Madrid, 28760 Tres Cantos, Spain; 4) Laboratory of Malaria and Vector Research, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20822.

Invasion of host cells by Plasmodium is a rapid process that begins with an initial weak interaction of the merozoite with the red-blood cell (RBC), followed by its apical re-orientation. The formation of a tight junction between the apical end of the merozoite and the RBC commits its entry into the RBC. This is mediated through the interaction of parasite surface AMA1 and its receptor RON2 secreted onto the host cell during invasion. Recent studies have shown that small molecules targeting AMA1 - RON2 interaction can successfully block merozoite invasion in a strain transcendent manner. Here, we screened the Tres Cantos Anti-Malarial Set (TCAMS) for inhibitors of AMA1-RON2 interaction. This library contains a diverse set of over 13,500 drug-like and lead-like compounds as a model, we found that the MAPK ERK7 is essential for correct biogenesis of the apical complex. Without active ERK7, parasites fail to develop mature ciliates, the organizing core of the Toxoplasma apical complex. Furthermore, we found that the apical cap protein, AC9, acts as a regulatory scaffold that controls ERK7 localization and activity. These discoveries provide a foundation to begin to dissect the regulation of apical complex biogenesis and function. CryoET (electron tomography) and cryoFIB (focused-ion-beam) allow the elucidation of large macromolecular complexes in their native states in flash-frozen cells with intact membranes. We will share recent data harnessing this technology to define the structures of the apical complex actively engaged in secretion.
potency in combination with current antimalarials. The AMA1- RON2 interaction interface is highly conserved among all P. falciparum parasites and inhibitors targeting this interaction could serve as a novel means to fight the threat of drug resistance against front-line antimalarials.

62 Moving Junction Complexes: Promising Targets for Vaccination against Toxoplasmosis Rania Najm1,2, Margarida Ruivo2, Diana Marcela Penarete-Vargas6, Mathieu Gisset3, Martin Boulanger4, Hiba El Hajj5, Maryse Lebrun6 1) Department of Experimental Pathology, Immunology and Microbiology, American University of Beirut, Beirut, Lebanon, MS; 2) UMR 5235 CNRS, Université de Montpellier, Montpellier, France, MS; 3) Center for Infection and Immunity of Lille, Institut Pasteur de Lille, Lille, France, PhD; 4) Departments of Biochemistry and Microbiology, University Victoria, Victoria, Canada, PhD; 5) Department of Experimental Pathology, Immunology and Microbiology, American University of Beirut, Beirut, Lebanon, PhD; 6) UMR 5235 CNRS, Université de Montpellier, Montpellier, France, PhD.

Toxoplasmosis was recently classified as a neglected parasitic infection necessitating public health control. Infection is mainly attained orally through the consumption of bradyzoites contained in cysts. Thereupon bradyzoites invade enterocytes and convert rapidly to tachyzoites, responsible for acute infection. Under the control of host immune response, tachyzoites convert back to bradyzoites that sustain a lifelong chronic disease. In immunocompromised patients, chronic toxoplasmosis may become fatal if left untreated. Invasion of Toxoplasma involves the formation of a tight connection between the parasite and the host cell membranes, referred as Moving Junction (MJ). In tachyzoite, the MJ is shaped by the assembly of a microneme protein AMA1 and a rhoptry neck protein RON2, as part of a complex involving additional RONs. Whilst the MJ process was well characterized in tachyzoites, invasion and MJ formation in other stages remain underexplored. Here, we showed that MJ proteins of tachyzoites are highly expressed and present at the MJ of invading bradyzoites. We also characterized further some paralogs of MJ proteins, barely expressed in tachyzoites. We found AMA2, AMA4 and RON2L1 highly expressed in bradyzoites and AMA4 at MJ of invading bradyzoites.

In light of the absence of an approved vaccine against human toxoplasmosis and the promises of a vaccine strategy against Plasmodium based on immunization with AMA1-RON2 complex, we explored the potential protection against toxoplasmosis by targeting MJ complexes. Immunization with recombinant AMA1 protein alone or in complex with a RON2 peptide, revealed a greater protective efficacy of the complex, both after oral challenge of mice with bradyzoites (>80% reduction of cysts burden) or intraperitoneal challenge with a lethal dose of tachyzoites (>80% mice survival). In accordance with its low expression in tachyzoites, AMA4 in complex with its binding RON2L1 peptide did not protect against acute infection, but selectively protected against chronic infection, supporting its role in the invasion of bradyzoites. Finally, IgG against either complexes inhibited MJ formation and invasion in vitro, suggesting that inhibition of invasion may be one plausible mechanism for in vivo protection.

Our results demonstrate that bradyzoites form a MJ during invasion, but with a different protein composition and validate the different MJ complexes as potential vaccine candidates to protect against toxoplasmosis.

63 Blocking Plasmodium falciparum sporogonic development in the mosquito with human-derived single-chain antibodies. Anna Maria Weyrich1, Maria Pissarev1, Rajagopal Murugan2, Gianna Triller2, Eric Marosi3, Giulia Costa1, Hedda Wardemann2, Elena A. Levashina1 1) Max Planck Institute for Infection Biology, Berlin Germany; 2) German Cancer Research Center, Heidelberg, Germany; 3) UPR9022 CNRS, U963 Inserm, University of Strasbourg, France.

The circumsporozoite protein (CSP), the major surface protein of P. falciparum (Pf) sporozoites, is a prominent target of malaria vaccines. Sporozoites are the only forms of the malaria parasite that are infectious to the human host upon a mosquito bite. CSP plays multiple roles during the parasite development, it is essential for sporogonic stages of parasite development within the mosquito midgut, for parasite protection from the cytotoxic SPEC2 protein in the human skin and for invasion of the human liver cells. However, how CSP mediates such diverse processes remains poorly understood.

In this project, we are exploiting the power of human-derived monoclonal antibodies recognizing the central repeat region of P. falciparum CSP (PfCSP). Expressed in different mosquito tissues we aim to disrupt several processes during the parasite journey in the mosquito Anopheles gambiae. We generated transgenic mosquitoes expressing a single-chain version of the antibodies directed against the repeat region of PfCSP in the mosquito fat body and the salivary glands. These transgenic mosquitoes successfully inhibited the development of a rodent malaria parasite P. berghei expressing PfCSP (PbCSP). Importantly, anti-CSP antibodies produced in the fat body completely abolished PbCSP sporogonic development in the oocyst, whereas the same antibodies expressed in the salivary glands inhibit parasite transmission to the next host. Hereby, we could show how anti-repeat antibodies targeting the oocyst are the most promising tool for the design of malaria-proof mosquitoes.

64 TcTASV antigens delivered in Baculovirus confer protection against Trypanosoma cruzi infection, notably reducing levels of circulating trypomastigotes, parasite tissue load and mortality. Yamil Ezequiel Masip1, Guido Molina2,3, Lucas Ceairo1,3, María Paula Molinari2,3, Valeria Tekiel1,3 1) Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo Ugalde", IIBIO (UNSAM - CONICET), Argentina; 2) Laboratorio de Baculovirus, Instituto de Biotecnología, INTA, Argentina; 3) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

TcTASV is a medium size multigenic family unique to the Trypanosoma cruzi present in all strains of the parasite and expressed in the life cycle stages of the mammalian host. Subfamilies TcTASV-A and TcTASV-C are the most numerous, are in contact with the host immune system and show differential expression patterns: TcTASV-A is expressed intracellularly in amastigotes and trypomastigotes while TcTASV-C is expressed at trypomastigote surface and secreted (Garcia et al, 2010; Bernabó et al, 2013; Floridia et al, 2016, 2019). Previous vaccine assays with TcTASV-C resulted in delayed appearance of bloodstream trypomastigotes but impacted only slightly in mortality, after challenge with RA (TcVI), a highly virulent T. cruzi strain. The immune response was essentially humoral, with negligible cellular response (Caeiro et al, 2018). We hypothesized that a vaccination protocol with TcTASV could be improved by triggering also a cellular response against TcTASV-A (intracellular antigen). As heterologous antigens displayed at baculovirus (BV) capsid have been reported to induce cellular responses, we engineered a recombinant BV that accurately express TcTASV-A (BV-TcTASV-A) fused to VP39, the major nucleocapsid protein. Mice were first immunized with TcTASV-A and then boosted with BV-TcTASV-A plus TcTASV-C. This immunization scheme induced a strong anti-TcTASV-A humoral response a

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Background: Cystic echinococcosis, caused by the cestode Echinococcus granulosus, is a neglected tropical disease with remarkable morbidity in humans and a problem of worldwide economic importance in livestock industry. Understanding the molecular basis of the parasite growth and development is essential for the disease diagnosis, management and control. The tetraspanin (TSP) family of proteins are transmembrane proteins with a role in many physiological processes of eukaryotic organisms. TSPs present in the tegumental surface of platyhelmiths play pivotal roles in host-parasite interaction. However, little is known about the role of TSPs in growth and development in the Platyhelmiths. To understand the role of TSP1 in the growth and development of E. granulosus we investigated the effect of EgTSP1-specific long dsRNA in different in vitro stages of the parasite.

Methods: Different stages of E. granulosus, protoscoleces and strobilated worms, were cultivated In vitro in di-phasic media. Using long dsRNA and two delivery methods, i.e. electroporation and electro-soaking, EgTSP1 silencing was performed with an EgTSP1-specific dsRNA. The TSP1 expression profile was assessed as well as the biological and ultrastructural properties of the parasites.

Results: After three days of dsRNA treatment, EgTSP1 expression was significantly reduced in both stages of E. granulosus as compared to irrelevant/unrelated dsRNA treated controls. Silencing expression of EgTSP1 in different stages of E. granulosus resulted in reduced viability and body contractions, inhibition of protoscoleces evagination and distinctive tegumental changes. Ultrastructural morphology of the strobilated worms treated with EgTSP1-specific dsRNA was indicative of the microtubres impairments and vacuolated tegument compared to the control helimits.

Conclusions: Results of the present study suggest that EgTSP1 plays important structural roles in tegument configuration in E. granulosus. EgTSP1 is proved to be a potential target for the development of vaccines and RNAi-based drugs.

66 The parasite coat protein VSGsur binds suramin to confer drug resistance Johan Zeelen1, Monique v. Straaten1, joseph Verdi1, Alexander Hempelmann1, Hamidreza Hashemi1, Kathryn Perez2, Philip D. Jeffrey3, F. Nina Papavasiliou1 1) Deutsches Krebsforschungszentren; 2) European Molecular Biology Laboratory Heidelberg; 3) Princeton University, Princeton, New Jersey, USA.

Suramin has been a primary early-stage treatment for African trypanosomiasis for nearly one hundred years. Recent studies revealed that trypanosome strains expressing the Variant Surface Glycoprotein VSGsur possess heightened resistance to suramin. We will show that VSGsur binds suramin with high affinity, whereas other VSGs do not.

Crystal structures of VSGsur and VSG13 will be presented, which identify a subfamily of VSGs that structurally diverges from all those resolved to date in several aspects, including a cavity between the monomers in the VSG-homodimer interface. VSGsur, and VSG13 harbor an N-linked glycan approaching the top of the molecule, positioned just under the β-sheet head and not in the bottom lobe as in the other VSG structures.

The co-crystal structure of VSGsur soaked with suramin reveals that the chemically symmetric drug binds within the large cavity in the VSGsur homodimer asymmetrically, primarily through contacts of its central benzene rings. Structure-based, loss-of-contact mutations in VSGsur significantly decrease the affinity to suramin and lead to a loss of the resistance phenotype.

Altogether, these data show that the resistance phenotype is dependent on the binding of suramin to VSGsur, establishing that the VSG proteins can possess functionality beyond their role in antigenic variation.

67 Whole-cell phenotypic screening of MMV pathogen box unravels new small molecules affecting late-stage development of plasmodium Alok Patra1, Rajesh Chandramohanadas2, Zbynek Bozdech2, Dhanasekaran Shanmugam3 1) Singapore University of Technology and Design, Singapore; 2) Nanyang Technological University, Singapore; 3) CSIR-National Chemical Laboratory, Pune.

Given the continuing emergence of Plasmodium falciparum parasites resistant to frontantimalarials and other combinatorial chemotherapies, there is an urgent need to develop new antimalarial drugs, especially those with novel mechanisms of action. Here, we report a systematic, cellular phenotype-based antimalarial screening of the MMV Pathogen Box collection, which facilitated the identification of specific blockers of late stage intraerythrocytic Plasmodium falciparum maturation. First, from standard growth inhibition assays, we discovered 62 additional antimalarials (EC50 ≤ 10 μM) over previously known antimalarial candidates from Pathogen Box. A total of 90 potent molecules (EC50 ≤ 1 μM) were selected for evaluating their stage-specific effects during the intra-erythrocytic development of P. falciparum. None of these molecules had significant effect on ring-trophozoite transition, 10 molecules inhibited trophozoite-schizont transition, and 21 molecules inhibited schizont-maturation.

Whole-cell phenotypic screening of MMV pathogen box unravels new small molecules affecting late-stage development of plasmodium Alok Patra1, Rajesh Chandramohanadas2, Zbynek Bozdech2, Dhanasekaran Shanmugam3 1) Singapore University of Technology and Design, Singapore; 2) Nanyang Technological University, Singapore; 3) CSIR-National Chemical Laboratory, Pune.

Whole-cell phenotypic screening of MMV pathogen box unravels new small molecules affecting late-stage development of plasmodium Alok Patra1, Rajesh Chandramohanadas2, Zbynek Bozdech2, Dhanasekaran Shanmugam3 1) Singapore University of Technology and Design, Singapore; 2) Nanyang Technological University, Singapore; 3) CSIR-National Chemical Laboratory, Pune.

The rapid insurgence of resistance to both insecticides and front-line antimalarial drugs threatens a global resurgence of malaria and makes the generation of effective tools for controlling this disease an urgent public health priority. To address this need, we recently demonstrated that the transmission of Plasmodium falciparum to Anopheles gambiae can be rapidly and completely blocked when female mosquitoes take up the antimalarial atovaquone from treated surfaces up to 24 hours before, or 12 hours after P. falciparum infection. Here, we expand on this proof of concept, demonstrating that the transmission blocking efficacy of mosquito antimalarial contact is unaffected when highly insecticide resistant, field-derived Anopheles mosquitoes are exposed to atovaquone. We further demonstrate that this approach can prevent transmission of diverse P. falciparum isolates from Burkina Faso and Cambodia, including an artesiminin resistant Kelch13 C580Y mutant parasite. We also demonstrate that, when mosquito exposure to antimalarials occurs after oocyst formation, parasite sporogony is arrested; delaying, or preventing, the arrival of sporozoites to the mosquito salivary glands. Finally, we show that mosquito ingestion of an antimalarial sugar solution can also block parasite transmission and sporogony. Taken together, these data suggest that targeting Plasmodium directly within the Anopheles vector would be an effective approach for malaria control, particularly in regions where extreme insecticide resistance has rendered conventional tools ineffective, and could be used in specific and highly targeted interventions to contain the spread of drug resistant parasites.

69 Chemogenomic approach to identifying nematode chemoreceptor drug targets in the entomopathogenic nematode Heterorhabditis bacteriophora Reeham Motaher1, Emilia Grill1, Andrea Langeland1, John Hawdon1, Damien O’Halloran1 1) George Washington University.
Parasitic nematodes cause diseases of major socioeconomic importance worldwide. Recent estimates indicate that over 1 billion people are infected with parasitic nematodes globally. Currently, parasitic nematode infections are controlled using anthelmintics in mass drug administration (MDA) practices. MDA protocols result in heavy anthelmintic exposure that risk developing resistant populations of nematodes. Several studies have already reported the emergence of anthelmintic resistant in human nematode parasites. Heavy exposure to anthelmintics has also selected populations of livestock parasitic nematodes that are no longer susceptible to certain anthelmintics in many areas around the world. The emergence of anthelmintic resistance in response to a small number of available drugs will inevitably generate more resistant human parasitic nematodes. Therefore, it is of critical importance that new anthelmintics are developed before resistance becomes widespread in human parasitic nematode populations. G-Protein Coupled Receptors (GPCRs) represent an important target for many pharmaceutical interventions with approximately 35% of Food and Drug Administration (FDA) approved drugs targeting GPCRs. Nematode Chemosensory GPCRs (NemChRs) are unique to nematodes, and their expression has been shown to correlate with the onset of parasitism, making them ideal substrates for target-based drug discovery. Here we set out to identify NemChRs from *Heterorhabditis bacteriophora* that are transcriptionally active in parasitic stages inside the host, and to use these NemChRs in a reverse pharmacological screen to identify repurposed anthelmintics that impede parasitic development. Our results uncovered several NemChRs that we ranked based on the highest fold change in transcription after host activation. We performed homology modelling and molecular dynamics simulations of these NemChRs, and from these models conducted a virtual screening campaign to identify candidate drug targets which were then experimentally tested in bioassays. Taken together, our results identify and characterize a candidate NemChR drug target, and provide a chemogenomic pipeline for identifying nematode substrates.

70 Phenotypic characterization of trypanosomes cells treated with tetracyclic iridoid, ML F52 suppression of flagella attachment proteins **Georgina Djaneh1**, Thelma Tetteh1, Takuhiro Uto3, Frederick Ayetey4, Michael Amoa-Bosompem2, Faustus Azerigiyik2, Kofi Baffour-Awuah2, Kofi Kwoifie1, Alfred Appiah4, Tomoe Ohta2, Irene Ayi1, Shiro Iwanga2, Nobou Ohta2, Yukihiro Shoyama3, Mitsuko Ohashi3 1) Noguchi Memorial Institute for Medical Research, University of Ghana. Accra, GHANA; 2) Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, JAPAN; 3) Nagasaki International University, Sasebo, Nagasaki, JAPAN; 4) Centre for Plant Medicine Research, Mampong, GHANA.

Despite the recent advances in finding a safe, effective, and easy to use chemotherapy for Human African Trypanosomiasis (HAT) remains a challenging task. This condition underlines the urgent need for the development of new drugs for the treatment of HAT. We previously identified the anti-trypanosome activities of three novel tetracyclic iridoids; ML-2-3, Molucilin and ML-F52, isolated from *Morinda lucida* with IC50 values of 3.75±µM, 1.27±µM and 0.43±µM, respectively. Immunohistochemistry (IHC) study showed that the compounds significantly suppressed the expression of PFR-2, which proceeded to the events of cell cycle alteration and apoptosis induction. Scanning Electron Microscopy revealed the severe phenotype of the flagella detached from the body of the parasite. Here we present a phenotypic characterization of ML-F52 treated trypanosomes in detail with analyzing the expression levels of Flagellum Attachment Zone (FAZ) filament proteins, Coiled-coil 2-domain containing protein (CC2D) and Flagella Attachment Zone protein 1 (FAZ-1) by IHC and Western blot assays. Immunohistochemistry study showed that ML-F52 significantly suppressed the expression of CC2D after 12 hours of post treatment whilst FAZ-1 did not show any significant suppression. After 24 hours of post treatment, cell length and FAZ length decreased with the emergence of cell containing detached flagella as compared to the control. Also, ML-F52 caused multinucleated phenotype and an increase in number of cells with only one or no visible kinetoplast. Western blot assay showed that CC2D expression was reduced more than approximately 60% by 12 hours post treatment and approximately 80% by 24 hours post treatment. Our findings suggested that ML-F52 might significantly inhibit the development and function of the flagellum.

71 Development of azithromycines analogues with dual-modality antimarial activity. Amy L. Burns1, 9, Brad E. Sleebs2, 3, Ghizal Siddiqui4, Amanda E. De Paoli4, Dovile Anderson4, Benjamin S. Liffner1, Richard Harvey1, James G. Beeson5, 6, 7, Darren J. Creek4, Christopher D. Goodman6, Geoffrey I. McFadden8, Danny Wilson1, 5 1) Research Centre for Infectious Diseases, School of Biological Sciences, the University of Adelaide, Adelaide, Australia.; 2) Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.; 3) Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia.; 4) Monash Institute of Pharmaceutical Sciences, Monash University, Victoria, Australia.; 5) Burnet Institute, Melbourne, Victoria, Australia.; 6) Department of Medicine, University of Melbourne, Parkville, Australia.; 7) Central Clinical School and Department of Microbiology, Monash University.; 8) School of Biosciences, University of Melbourne, Parkville, Victoria, Australia.; 9) Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA.

Background: Resistance to front-line antimalarials is spreading, and development of new drug treatment strategies to rapidly kill *Plasmodium* spp. parasites is urgently needed. Azithromycin is a clinically used macrolide antibiotic proposed as a partner drug for combination therapy in malaria. However, its slow-killing ‘delayed death’ activity against the parasite’s apicoplast organelle and suboptimal activity compared to alternative therapies limit its applications for clinical use. Here, we explore a panel of azithromycines analogues demonstrating that chemical modifications can be used to enhance the speed of antimalarial action and improve the potency of action.

Results: Investigation of 84 azithromycines analogues revealed nanomolar quick-killing potency directed against the very earliest stage of parasite development within red blood cells. Indeed, the best analogue exhibited 1600-fold higher potency than azithromycin with less than 48 hours treatment *in vitro*. Analogues were effective against the zoonotic malaria parasite, *P. knowlesi*, and against both multi-drug and artemisinin resistant *P. falciparum* lines. Metabolomic profiles of azithromycin analogue treated parasites suggested activity in the food vacuole and mitochondria were disrupted. Moreover, unlike the food vacuole targeting drug chloroquine, azithromycin and analogues were active across blood stage development, including merozoite invasion, suggesting that these macrolides have a multi-factorial mechanism of quick-killing activity. The positioning of functional groups added to azithromycin and its quick-killing analogues altered their activity against bacterial-like ribosomes but had minimal change on quick-killing activity. Apicoplast minus parasites remained susceptible to both azithromycin and its analogues, further demonstrating that quick-killing is independent of apicoplast-targeting, delayed-death activity.

Conclusion: We show that azithromycin and analogues can rapidly kill parasite asexual blood-stages via a fast acting mechanism of action that is independent of the apicoplast. Development of azithromycin and analogues as antimalarials offers the possibility of targeting parasites through both a quick-killing and delayed death mechanism of action in a single, multifactorial, chemotype.

72 IRON OXIDE NANO PARTICLES FORTIFIED ARTESunate – can we use a nanomedicine approach for warfare against malarial parasites? Deepika Kannan1, Nisha Yadav1, Shakeel Ahmed2, Bimlesh Lochab1, Shailja Singh2 1) Shiv Nadar University; 2) Jawaharlal Nehru University.

The daunting challenge of malaria elimination has been prevailing since ages. More than a decade, with widespread occurrence globally, the emergence of Artemisinin resistance has been dismaying. The failure in combating the disease may be attributed to the gain of drug resistance, limited efficacy of available drugs, or lack of immunogenicity of vaccine antigens against all the strains of plasmodium. Therefore, here we aim for an innovative biocompatible nanomedicine approach to boost the efficacy of the existing potential iron-dependent anti-malarial drug artemesunate. The supplementation of free iron in the case of malarial patients has always been controversial in terms of lethality with studies depicting the beneficial effect of iron supplements in patients suffering from iron-deficient anemia and severe malaria-associated anemia. Controversially, results from other studies indicated a failure to clear malaria in a large proportion of people due to high levels of parasitemia when supplemented iron with antimalarials. However, in all these studies, iron supplements have been the source of ferrous iron (Fe2+). Within the erythrocytes, the malarial parasite is known to metabolize hemoglobin for nutrient source and utilizes intraerythrocytic labile pool as an iron source. Therefore, the provision of iron supplements in the active form (Fe2+) may result in increased bioavailability of iron within the labile pool.

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favoring the rapid growth of the parasite. Herein, we explored for the first time the capacity of surface coated iron oxide NPs (IONPs) to enhance the efficacy of the malarial drug Artesunate. A combination of surface coated iron oxide nanoparticle with Artesunate enabled enhancement in the efficacy of Artesunate radically by ~8-10 fold both in vivo and in vitro. The internalization of IONPs within the parasite food vacuole, a low pH environment, provided the best combination for the slow conversion of ferric to ferrous ions enabling the sustainable release of the ferrous ions, persistent activation of Artesunate and pro-long generation of radical species via endoperoxide cleavage. In line with this, combination-treated parasites observed heightened DNA and protein damage. Furthermore, the surface coating of ATA-IONP has inverse toxic effects of iron both in vivo and in vitro, inhibiting the availability of ferrous ions for the parasite growth. Also, the combination of IONPs with artemesin proved efficacious against Artemisinin resistant parasites-R539T. This is congruous with the currently known theory behind ART resistant parasites, with K13 mutated parasites depicting reduced hemoglobin uptake & metabolism, averting the drug action. Henceforth, the fabrication of the nanomedicine advocates a strategic approach to eliminate the parasite load sustainably and to potentiate ART activity against sensitive & drug-resistant parasites.

73 Discovery of a novel antiparasitic compound that promotes leishmanial tubulin polymerization Imran Ullah1, Suraksha Galahawati1, Laela Booshehri1, Hanspeter Niederstrasser1, Shreoshi Majumdar1, Christopher Leija1, James Bradford1, Bin Hu1, Joseph Ready1, Dawn Wetzel1 1) UT Southwestern Medical Center.

There is a significant unmet clinical need to discover new drugs to combat protozoan infections. To identify new antileishmanial compounds, we screened the Medicines for Malaria Venture “Pathogen Box” for activity on Leishmania amazonensis axenic amastigotes. We found six antileishmanial hits that had EC50 values between 50 - 480 nM. Concentration-response assays showed that our best hit, MMV676477, had potent activity against axenic and intracellular Leishmania amastigotes, Plasmodium falciparum, and Trypanosoma brucei. We next synthesized analogs of MMV676477 to explore structure–activity relationships (SAR) for Leishmania amastigotes, and we obtained a wide activity range (20 - 5000 nM). SW41, our most active analog, had five-fold improved antileishmanial potency compared to the parent compound. Our target identification studies indicated that MMV676477 selectively stabilized Leishmania microtubules. We found that active MMV676477 analogs affected L. amazonensis morphology and increased the percentage of parasites undergoing cell division. Differential centrifugation of parasite lysates demonstrated that MMV676477 promoted tubulin partitioning towards the polymeric form. Turbidity assays using purified leishmanial or porcine tubulin showed that MMV676477 preferentially promoted Leishmania tubulin polymerization. Active analogs’ ability to stimulate Leishmania tubulin polymerization correlated with their antiparasitic activity. Finally, chemical cross-linking studies demonstrated that the MMV676477 scaffold bound purified leishmanial tubulin, and competition assays correlated binding of compounds to tubulin with antileishmanial activity. In total, our studies have identified MMV676477 as a potent antiparasitic compound that preferentially promotes polymerization of Leishmania microtubules. Due to its broad-spectrum antiparasitic activity and selectivity, our scaffold shows significant promise for future antiparasitic drug development efforts.

74 De novo synthesis of phosphatidylcholine is essential for the promastigote but not amastigote stage in Leishmania major Somarat Moitra1, Somrita Basu1, Mattie Pawlowicz2, Fong-Fu Hsu3, Kai Zhang1 1) Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA; 2) Wellcome Centre for Anti-Infective Research(WC AIR), University of Dundee, Dundee, DD1 5EH, Scotland, UK; 3) Division of Endocrinology, Metabolism & Lipid Research, Washington University School of Medicine, Saint Louis, MO, USA.

Phosphatidylcholine (PC) is the most abundant group of phospholipids in eukaryotes constituting 30–35% of total lipids in Leishmania. PC synthesis is mainly occur via the choline branch of the Kennedy pathway (choline → choline-phosphate → CDP-choline → PC) and the N-methylation of phosphatidylethanolamine (PE). In addition, Leishmania parasites can also acquire lipids from the host or culture medium. Our previous study on the choline-phosphate cytididylytransferase (CPT) demonstrates that the formation of CDP-choline from choline-phosphate and CTP is dispensable for the promastigotes and amastigotes of Leishmania major. Thus, these parasites may bypass the CDP-choline pathway of PC and C16:0–C20:0 acylglycerol production, PE metabolism and lipid salvage. In this study, we assessed the function and essentiality of choline ethanolamine phosphotransferase (CEPT) which is directly responsible for the de novo synthesis of both PC and PE. This is important because in addition to being principle membrane components, PC and PE are precursors for a number of vital intermediates including diacylglycerol, lysophospholipid and phosphatidic acid. Understanding how Leishmania generate PC and PE may reveal new ways to block their growth. Our data indicate that L. major CEPT is localized in the ER and possesses the activity to synthesize PC from CDP-choline and diacylglycerol. Targeted deletion of CEPT is only possible in the presence of an epimonal CEPT in the promastigote stage of L. major. These chromosomal null parasites require the epimonal expression of CEPT for survival in culture, confirming its essentiality during the promastigote stage. In contrast, during in vivo infection of BALB/c mice, these chromosomal null parasites appeared to lose the epimonal copy of CEPT while maintaining a normal level of virulence, replication and cellular PC. Therefore, while the de novo synthesis of PC/PE is indispensable for proliferation of promastigotes, intracellular amastigotes could acquire the majority of their lipids from the host.

75 Gut metabolites influence susceptibility to Cryptosporidium infection Lisa Funkhouser-Jones1, Marianna Akey1, Georgia Wilke1, Kelli VanDusen2, Deborah Schafer3, Kevin Ackman3, Michael Riggs3, Thaddeus Stappenbeck4, L. David Sibley1 1) Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO; 2) Department of Pediatrics, Divisions of Gastroenterology, Hepatology, and Nutrition of Developmental Biology, University of Cincinnati College of Medicine and the Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) Department of Biomedical sciences, Iowa state university.

Cryptosporidium is a leading cause of diarrheal disease in infants and toddlers in resource-poor localities but is not commonly observed in children over two years of age. Neonatal animals in general are highly susceptible to human cryptosporidiosis. Over two years of age. Neonatal animals in general are highly susceptible to human cryptosporidiosis. and an air-liquid interface (ALI) transwell culture system. Our findings demonstrate that medium and long-chain saturated fatty acids inhibit the growth of C. parvum invasion and growth in an adenocarcinoma cell line (HCT-8) and an air-liquid interface (ALI) transwell culture system. Our findings demonstrate that medium and long-chain saturated fatty acids inhibit C. parvum growth, while long-chain unsaturated fatty acids enhance C. parvum invasion. The influence of these two classes of metabolites on C. parvum infection likely reflects the streamlined metabolism in C. parvum, which is unable to synthesize fatty acids. In addition, we screened a metabolite library derived from adult mouse microbiota and found several classes of compounds that significantly inhibited, but none that enhanced, the growth of C. parvum in vitro. Hence, gut metabolites contribute to the differences in susceptibility to Cryptosporidium observed in neonatal versus adult animals and could play a role in enhancing, or preventing, human cryptosporidiosis.

76 Accessible Cholesterol in the Erythrocyte Plasma Membrane is Essential for P. falciparum Invasion and Growth Avantika Ahiyai1, Suyash Bhatnagar1, Joanne Morrisey1, Josh Beck2, Akhil Vaidya1 1) College of Medicine, Drexel university; 2) Department of Biomedical sciences, Iowa state university.

We have recently shown that a wide range of aldehydes targeting two different transmembrane proteins (PTATP4 and PNCR1) cause rapid disruption of cholesterol homeostasis in P. falciparum. Neither the parasite nor its host RBC is capable of cholesterol synthesis, and thus proper dispensation of this important lipid requires redistribution of cholesterol that was endowed to the RBC at the time of its maturation. Here we report that depletion of accessible

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cholesterol from the RBC plasma membrane by methyl-β-cyclodextrin (MBCD) has dramatic consequences, resulting in an inability of the parasite to invade RBC as well as in inhibition of the parasite growth. These defects were complemented by reconstitution with cholesterol or epicholesterol but not with desmosterol. These results suggest an important role for the aliphatic portion of the sterol, but not the polar group, in parasite invasion and growth. Using live time-lapse videography of fluorescently tagged trophozoite stage parasites, we detected rapid expulsion of the parasite when exposed to MBCD for just 30 min. The propeller trophozoites were still surrounded by parasitophorous vacuolar membrane (PVM) while remaining tethered to intact RBCs. Electron microscopy revealed the PVM to be compromised in the extruded parasites. Remarkably, prior 2 h treatment with PIAT4P or P1NCR1 inhibitors prevented the extrusion of trophozoites when exposed to MBCD. Overall, these findings suggest a dynamic movement of accessible cholesterol within P. falciparum-infected RBC that is critical for parasite survival. It would be fruitful to explore molecular players participating in this hitherto unknown aspect of parasite physiology.

77 Plasmodium berghei Sporozoites in Nonreplicative Vacuoles Are Eliminated by a PI3P-mediated Autophagy-independent Pathway Annina Bindschedler1,2, Rahel Wacker1,2, Jessica Egli1, Nina Eickel1,2, Jacqueline Schmuckli-Maurer1, Blandine M. Franke-Fayard3, Chris J. Jansei3, Volker Heussler1 1) Institute of Cell Biology, University of Bern, Bern, Switzerland; 2) Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland; 3) Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands.

Plasmodium sporozoites infect hepatocytes, where they undergo a first round of asexual replication. Before they invade a hepatocyte by generating a parasitophorous vacuole membrane (PVM), the parasites transmigrate through several cells. Transmigration can be achieved either by cell wounding or by the formation of a nonreplicative transient vacuole, which markedly differs from the PVM in its molecular composition. Using SPECT2-deficient parasites, which are not able to survive from transient vacuoles during cell traversal, we could show that parasites trapped in their nonreplicative transient vacuole provoke a host cell response that clearly differs from the previously described Plasmoid-associated autophagy-related (PAAR) response. In PAAR response we observe a long-lasting association of the autophagy marker protein LC3 with the PVM, which is not preceded by phosphorylation of 3-phosphate (PI3P) labelling. In contrast, parasites residing in a nonreplicative transient vacuole provoke PI3P-labelling of the vacuolar membrane immediately after invasion followed by a transient LC3-labelling and elimination through lysosomal acidification. Thus, host cells can employ a pathway distinct from the previously described PAAR response to efficiently recognize and eliminate parasites.
Membrane lipid synthesis and remodeling are key processes that support proliferation and developmental transitions in the kinetoplastid protozoan parasite *Trypanosoma cruzi*. The importance of endogenous fatty acid (FA) synthesis in this parasite remains unclear due to the capacity of the proliferative stages to take up exogenous FA from the environment. In the related parasite, *Trypanosoma brucei*, it has been shown that the bulk of FA synthesis is carried out by the elongase (ELO) system. Distinct from type I or II fatty acid synthesis pathways, the ELO pathway consists of four enzymes (ELO1-4) that sequentially elongate short-chain fatty acids (SCFA) to long- or very-long-chain fatty acids (LCFA or VLCFA). The first enzyme in the pathway, ELO1, converts C4:0 to C10:0. ELO2 extends C10:0 to C14:0. ELO3 elongates C14:0 to C18:0, and ELO4 synthesizes very-long-chain and polyunsaturated fatty acids. To study the role of FA elongases in regulation of membrane lipid composition in *T. cruzi*, we generated parasites with functional disruptions in individual elo1, elo2 and elo3 genes and their cognate genetically complemented lines. Initial characterization of epimastigote (EPi; proliferative, extracellular insect stage) lipids by high-resolution mass-spectrometry (HR-MS)-based lipidomics revealed a decrease in the hydrophobicity of lipids extracted from the ELO-deficient parasites as compared to WT, suggesting the usage of SCFAs (C10:0, C12:0, C14:0, C14:1) when LCFA (C16:0, C18:0, C18:1, and C18:2) synthesis is impaired. Quantitative HR-MS lipidomic analysis of Δelo3 parasites confirmed this result. Δelo1 Epis, but not Δelo2 or Δelo3, have a severe growth defect suggesting an importance for endogenous SCFA synthesis in this stage. All of the mutants are competent to establish infection in mammalian cells; however, trypanomastigote infectivity and the intracellular amastigotes growth of Δelo2 and Δelo3 mutants is markedly reduced, implying a greater reliance on endogenously synthesized LCFA in these life cycle stages. Biochemical supplementation fails to restore normal growth in either proliferative stage. Combined, our data suggest that despite previous demonstrations of lipid and fatty acid scavenging by *T. cruzi* 2, exogenously fed FAs do not entirely compensate for the loss of endogenous LCFA synthesis in the Δelo mutants, supporting the role for the ELO pathway in maintaining homeostatic control of membrane lipid composition in *T. cruzi*.


81 Characterization of Toxoplasma gondii acyl-CoA synthetases reveals the critical roles in lipid synthesis and parasite development of TgACS1 and TgACS3
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Apicomplexa parasites, *T. falciparum* and *T. gondii*, cause major human diseases affecting a third of the global population. Their prevalence and drug resistance emphasize the need to identify novel drug targets. Current evidence shows that lipid synthesis and trafficking are pertinent metabolic pathways enabling host-parasite interactions and parasite survival. Fatty acids (FA) are critical metabolites for lipid synthesis and parasite propagation. The utilization of FA requires a metabolic activation step. This step can be mediated by acyl-CoA synthetases (ACSs) that activate FA by binding it to a Coenzyme A molecule, forming Acyl-CoAs. The role of these enzymes in FA utilization in *T. gondii* has not been fully elucidated. We thus sought to assess their functions in *T. gondii* where we identified 7 putative ACSs encoded in the genome of the parasite. Interestingly, we determined their physiological localizations to different sub-cellular compartments of the parasite, suggesting exclusive functions. We identified the most crucial ones during tachyzoite life stages and performed detailed cellular and lipidomic characterization of key ACSs. Briefly, TgACS1 and TgACS3 are both critical for intracellular development and act differently depending on host nutritional environment. TgACS1 is a cytosolic/vesicular protein that has specific roles in lipid droplet formation, and lipid storage utilization during intra- and extracellular life stages. Its disruption particularly affects motility and energy storages during extracellular life stages. TgACS3, a perinuclear/vesicular protein regulates replication and growth of intracellular Toxoplasma tachyzoites. Lipidomic analyses of parasites lacking TgACS3 reveals its role in activation of FAs directed towards parasite phospholipid synthesis and membrane biogenesis. Our data further our understanding of the activation and usage of FAs in Apicomplexa parasites, which are essential to their survival. This may contribute to identifying new drug targets in the combat against malaria and toxoplasmosis.

82 Variant Surface Glycoprotein Expression in Tissue-resident *Trypanosoma brucei*
Alexander Beaver1, 2, Lucy Zhang, Bryce Bobb, Filipa Rijo-Ferreira3,4,5, Luisa Figueiredo 3, Monica Mugnier1,2 1) Johns Hopkins School of Medicine, Baltimore, MD; 2) Johns Hopkins School of Public Health, Baltimore, MD; 3) Instituto de Medicina Molecular, Universidade de Lisboa, Lisboa, Portugal; 4) Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas; 5) Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

The extracellular parasite *Trypanosoma brucei* evades the host immune system by a process of antigenic variation in which the parasite continuously changes its variant surface glycoprotein (VSG) coat to avoid recognition by host antibodies. Previous studies have shown that during an infection parasites occupy the blood and several extracellular spaces, including the adipose tissue, skin, and lungs. Currently, little is known about VSG expression and diversity in these extracutaneous parasites. Using VSG-Seq to examine VSGs expressed by parasites in the blood and tissues of infected mice, we found that populations of parasites in the tissues, specifically the gonadal fat, expressed a greater diversity of VSGs when compared to parasites in the blood. Despite this greater diversity of VSGs in parasites in tissues, parasites in different mice and tissue spaces often expressed the same VSGs, suggesting that a switching hierarchy still influences VSG expression. In addition, the initiating VSG was often still detectable in the tissues after it had been completely cleared from the blood, hinting at a difference in the host immune response between the blood and tissues. These results suggest that *T. brucei* VSG expression is shaped by both a hierarchy of preferred VSGs and by tissue-dependent environmental pressures.

83 Trypanosomes having pHun: cyclic AMP signalling in perception and response to pH gradients
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*Trypanosoma brucei* spp colonise a variety of tissues in their insect and mammalian hosts. At present, virtually nothing is known about what drives trypanosomes to leave one tissue compartment and enter another. Social motility (SoMo), the coordinated migration and self-organising properties of procyclic trypanosomes on agarose plates, has the potential to offer insights into this process. Previous studies indicated that early procyclic forms release and respond to pH gradients. We investigated members of three protein families, adenylate cyclases (ACs), phosphodiesterases (PDEs) and cAMP response proteins (CARPs) and evaluated their importance for SoMo and pH taxis. Mutants of AC5 and CARP3 show a SoMo defect and are not repelled by acid, but are still attracted to alkali. A pulldown confirmed that there is a physical interaction between CARP3 and AC5. In addition, a PDE1 knockout, which was previously shown to have a major SoMo defect, and struggled to traverse the peritrophic matrix in the tsetse fly, does not respond to pH at all. Our data are compatible with pH-taxis and self-generated pH gradients being components of social motility. These findings strengthen our hypothesis that cAMP signalling is required to sense and move along
chometactic gradients and raise the possibility that tissue tropism within mammals is due, at least in part, to chemotaxis. The physiological relevance, in the context of trypanosome metabolism and pH gradients within the tsetse host, will be discussed.

84 Fatty acid- and retinol-binding proteins secreted by parasitic nematodes dampen host immune responses by interfering with host lipid signaling mechanisms Sophia Park1, Chau Nguyen1, Shyon Nasrolahi1, Damian Juncaj1, Dihong Lu1, Raghavendran Ramaswamy22, Anna Buchman3,4, Omar Akbari3,4, Naoki Yamanaka5, Martin Boulanger2, Adler Dillman1 1) Department of Nematology, University of California, Riverside, California; 2) Department of Biochemistry and Microbiology, University of Victoria, BC, Canada ; 3) Division of Biological Sciences, Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, California; 4) Tata Institute for Genetics and Society, University of California, San Diego, La Jolla, California; 5) Department of Entomology, University of California, Riverside, California.

Parasitic nematodes cause many diseases on a global scale, yet little is known about how they are able to infect hosts and evade their immune systems. One hypothesis is that proteins and small molecules released by these parasites interfere with host immune functions. The fatty acid- and retinol-binding (FAR) protein family are thought to be involved manipulating host immunity. The mechanism and functional role of FARs during active infection in an animal host is unknown. A major difficulty in studying nematode host-parasite interactions is the lack of model systems that are safe and relevant to a wide variety of infections. We have leveraged *D. melanogaster* as our model host along with the generalist insect-parasitic nematode *Steinernema carpocapsae*. *S. carpocapsae* releases two FARs in high abundance during an active infection and we have tested the immunomodulatory effects of these by generating transgenic flies that express the protein as well as by exposure to recombinant proteins to more closely analyze dose dependent effects. The function of FARs was evaluated in the context of a bacterial infection. We have found that nematode FAR proteins modulate host immunity during a bacterial infection by decreasing host resistance and inhibiting various immune functions. We also found that FARs lead to a decrease in eicosanoid signaling molecules *in vivo* and bind to these compounds *in vitro* suggesting that FARs modulate immunity by altering the availability of immune signaling molecules. The FAR protein family is highly conserved among nematodes and understanding the molecular interactions of FAR could lead to major advances in treating nematode infections.

85 The fibrinolytic system enables the onset of *Plasmodium* infection in the mosquito Thiago Silva1, Tales Pascini1, Zarna Pala1, Patricia Alvarenga1, Yeong Jeong1, Janet Olivas1, Hanhuy Bu2, Marcelo Jacobs-Lorena2, Joel Vega-Rodriguez1 1) National Institute of Health; 2) Johns Hopkins University.

*Plasmodium* spp. must migrate across proteinaceous matrices to successfully infect the mosquito vector and the vertebrate host. While parasite motility is powered by a subpellicular actomyosin motor, the contribution of host factors to facilitate parasite migration is largely underexplored. Plasmin, the effector protease of mammalian fibrinolysis, degrades fibrin and other extracellular matrix proteins allowing cell migration through tissues. We previously reported that *Plasmodium falciparum* hijacks human plasmin for successful infection of the mosquito midgut, a crucial step for parasite transmission. However, the mechanism by which parasite-bound plasminogen is activated into plasmin and its molecular targets are unknown. Here, we report that inhibition of fibrinolysis arrests parasite development at the early stages of sexual reproduction inhibiting ookinete formation. Plasminogen bound to the gamete surface is processed into plasmin by the mammalian tissue-type-plasminogen activator that is co-recruited to the parasite surface. Furthermore, we show that the increase of fibrinogen concentration and fibrin polymerization in the blood bolus, both natural substrates of plasmin, inversely correlate with parasite infectivity of the mosquito. We propose that Plasmodium sexual stages hijack the mammalian fibrinolytic system to facilitate motility and development within the mosquito blood bolus. This is a new host-parasite interaction that can be targeted for transmission-blocking strategies.

Wednesday, September 23 2:00 PM - 4:05 PM

**Session 11B, Evolution and Genomics/Session 12B, Antigenic Variation**

86 Diverse evolutionary pathways thwart the use of collateral sensitivity as a strategy to suppress resistance for *Plasmodium* dihydroorotate dehydrogenase inhibitors Rebecca Mandt1, Maria Jose Lafuent-Monasterio2, Madeline Luth3, Sabine Otlie3, Elizabeth Winzeler3, F. Javier Gamo2, Dyann Wirth1,4, Amando Lukens1,4 1) Harvard T.H. Chan School of Public Health; 2) Tres Cantos Medicines Development Campus, GlaxoSmithKline; 3) University of California, San Diego; 4) Broad Institute.

Antimicrobial resistance threatens our ability to control many global infectious diseases, and malaria is no exception. One proposed strategy to suppress resistance is to take advantage of collateral sensitivity, in which resistance to one drug causes increased sensitivity to another. Here, we focus on resistance and collateral sensitivity to *Plasmodium* dihydroorotate dehydrogenase (DHODH) inhibitors, including the clinical candidate, DSM265. We previously identified 13 distinct point mutations in dhodh that confer resistance to DSM265. Interestingly, many of these mutations also cause increased sensitivity to DHODH inhibitors with distinct chemotypes. In screens of resistant parasites, we identified one molecule, TCMDC-125334, that was active against all DHODH mutant lines tested. Here, we characterize the *in vitro* evolution of resistance to TCMDC-125334. We find that resistance can be mediated by a novel point mutation, DHODH I263S, which confers 4-fold resistance to TCMDC-125334 but not DSM265. We next treated parasites with DSM265 and TCMC-125334 in combination. While resistance to the combination failed to emerge, we ultimately selected parasites with a single point mutation, DHODH V532A, that conferred cross-resistance to both compound (5-fold fold resistance to TCMDC125334; 18-fold resistance to DSM265). We further showed that DHODH V532A parasites were relatively fit in *in vitro* competitive growth assays. We therefore isolate this cross-resistant, competitively fit mutant line argues against the using this combination therapy approach to block the emergence of resistance. We also wanted to test what would happen if we selected a mutant line that was hyper-sensitive to TCMDC-125334. We used DHODH C276Y parasites, which are 10-fold more sensitive to TCMDC-125334 compared to wildtype. Previously, we found that in a similar selection with the DHODH E182D mutant line, parasites reverted back to wildtype upon treatment with a mutant-active inhibitor. We here found that DHODH C276Y parasites gained an additional DHODH F227Y mutation, which conferred high-level (>100-fold) resistance to DSM265 while decreasing sensitivity to TCMDC-125334. These data demonstrate that an initial mutation can open pathways to higher-level resistance. Further, the continued identification of mutations in DHODH, including multiple amino acid changes that occur at the same sites, highlights the flexibility of the DHODH enzyme and its liability as a drug target.

87 Genetic analysis of transmission stage production and virulence in schistosome parasites Winka Le Clec’h1, Frédéric Chevalier1, Marina McDew-White1, Vinay Menon1, Grace-Ann Arya1, Robbie Diaz1, Timothy Anderson1 1) Texas Biomedical Research Institute.

Both theory and experimental data suggest that the production of transmission stages should be strongly associated with parasite virulence, but the genetic basis of parasite transmission/virulence traits is poorly understood. In the blood fluke *Schistosoma mansoni*, parasite genotypes differ 7-fold in numbers of cercariae larvae shed from infected snails. Furthermore, high shedding parasites cause high mortality to snails while low shedding parasites cause low mortality, consistent with expected trade-offs between parasite transmission and virulence. This project was designed to understand the genetic basis of transmission stage production/virulence. We then conducted classical linkage mapping to reveal quantitative trait loci (QTLs) associated with transmission stage production. Cercarial production is polygenic: we found three major QTLs on chromosome 1, 3 and 5 (Log-of-the-odds (LOD) = 5.61, 8.19, 6.25) and two minor QTLs on chromosome 2 and 4. These QTLs act additively and explained 28.15% of the phenotypic variation observed in
cercarial shedding. Alleles inherited from the high and low shedding parents were co-dominant at all QTLs, except for chr. 1 where the “high cercarial shedding” allele is recessive. These results demonstrate that the genetic architecture of key traits directly relevant to schistosome ecology can be dissected using genetic crosses and linkage mapping, and sets the stage for fine mapping and functional validation of the genes involved using the growing armory of functional and cell biology tools available for this parasite.

88 Reevaluation of the Toxoplasma gondii and Neospora caninum genomes reveals misassembly, karyotype differences and chromosomal rearrangements

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Neospora caninum primarily infests causing abortions with an estimated impact of a billion dollars on worldwide economy, annually. However, the study of its biology has been unheeded by the established paradigm that it is virtually identical to its close relative, the widely studied human pathogen, Toxoplasma gondii. By revisiting the genome sequence, assembly and annotation using third generation sequencing technologies, here we show that the N. caninum genome was originally incorrectly assembled under the presumption of synteny with T. gondii. We show that major chromosomal rearrangements have occurred between these species. Importantly, we show that chromosomes originally annotated as ChrV1b and VIII are indeed fused, reducing the karyotype of both N. caninum and T. gondii to 13 chromosomes. We reannotate the N. caninum genome, revealing over 500 new genes. We sequence and annotate the non-photonsynthetic plastid and mitochondrial genomes, and show that while apicoplast genomes are virtually identical, high levels of gene fragmentation and reshuffling exist between species and strains. Our results correct assembly artifacts that are currently widely distributed in the genome database of N. caninum and T. gondii, but more importantly, highlight the mitochondrion as a previously overlooked source of variability and pave the way for a change in the paradigm of synteny, encouraging rethinking the genome as basis of the comparative unique biology of these pathogens.

89 Development of a computational workflow for functional variant detection in Plasmodium falciparum drug selections

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Whole genome sequencing of naturally or experimentally drug-selected parasites is a powerful tool for identifying alleles that confer drug resistance. Such alleles can serve as markers of drug resistance, or in the case of experimental compounds, may point to new druggable targets. However, given the number of mutations observed in a typical in vitro evolution or genome-wide association experiment, it may not be feasible to attempt functional validation via CRISPR-Cas9 for each one. To better predict functional variants, we developed a computational approach based on the concept of supervised learning and applied it to the Malaria Drug Accelerator (MaDA) Consortium’s repository of 88 compound selections and 464 lab-generated resistant clones. In addition to being the largest in vitro chemogenomic dataset available for the malaria parasite, the MaDA portfolio contains many functionally validated targets and resistance mechanisms that can be used as internal controls. We assigned categorical weights to the 2,637 unique mutations in the dataset based on characteristics like whether they were coding, noncoding, or intergenic; found in genes expressed during the asexual blood stage of parasite development; located in genes essential for Plasmodium growth/survival; within a member of a multigene family; a singleton versus one of multiple alleles in the same gene; or mapped to a functional domain of a protein. Based on empirically shared features among MaDA’s validated targets and resistance mechanisms (which include PICT, PFMDR1, PHTP4, PBDHOM, among others), gene essentiality and functional domain were the characteristics weighted most heavily. When scores were calculated across all mutations in the dataset, we pulled out multiple genes with target-like properties that to our knowledge have not yet been pursued in the context of antimalarial drug development, which could be interesting starting points.

90 The VEX-gression [VEX] complex orchestrates VSG allele-exclusive interactions with the spliced-leader locus in trypanosomes

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Trypanosoma brucei lacks classical sequences or regulated transcription initiation. However, it employs an enigmatic mechanism of monogenic antigen expression to evade the host immune response. The association of one of approximately fifteen telomeric variant surface glycoprotein (VSG) genes with an RNA-polymerase-I (pol-I) transcription factory facilitates singular VSG expression. Notably, efficient RNA processing and maturation are somehow restricted to the active-VSG, suggesting that the access to RNA processing factors or substrates might be limited. We recently identified a chromatin-associated VSG exclusion (VEX) complex containing VEX1 and VEX2; VEX2, in particular, is required to sustain VSG monogenic expression, but by an unknown mechanism (Faria et al, 2019; PMID: 31289266). We now show that the VEX-complex sustains allele exclusion by co-ordinating allele-exclusive inter-chromosomal interactions with an RNA maturation locus.

VEX1 or VEX2 ChiP-Seq analyses revealed an association not only with the single active-VSG but also with the spliced-leader (SL) array, a genomic locus that encodes for the SL-RNA, the key substrate for trans-splicing. DNA FISH and super-resolution microscopy revealed that only the active-VSG expression site (not the ‘silent’) was in close spatial proximity to the splicing locus in the 3D nuclear space; the association was dynamic during S phase but stably propagated through the cell cycle. Super-resolution microscopy also showed that VEX1 and VEX2 occupy the splicing and VSG expression site compartments, respectively.

To further investigate the role of the VEX-complex, we tracked VSG expression sites, SL-arrays and pol-I. Following VEX2 knockdown, the pol-I compartment separates from the splicing compartment and disperses, while previously ‘silent’ VSG expression sites cluster around the SL-arrays (DNA FISH) and are derepressed (RNA-Seq). Therefore, VEX2 emerges as an exclusion factor that allows only one VSG to access the splicing compartment at a time.

We found VEX2 to be a large (>200 kDa) multimeric RNA-helicase that forms a native complex of approximately 1 MDa. Notably, a family of helicases were recently shown to be global regulators of RNA-containing, phase-separated sub-nuclear organelles. Indeed, the VEX2 compartment is specifically disrupted following treatment with 1,6-hexanediol, which suppresses liquid-liquid phase-separation. We observe similar disruption of the VEX2 compartment following inhibition of transcription or splicing, suggesting that RNAs from the active-VSG expression site are required for the formation of VEX2 condensates.

Our results reveal a novel VEX2-dependent mechanism that ensures both monogenic transcription and efficient RNA processing through the spatial integration of antigen transcription and mRNA splicing in a dedicated compartment.

91 Trypanosoma brucei RAP1 has an RNA binding activity that is essential for VSG monoallelic expression

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Telomeres are nucleoprotein complexes located at chromosome ends and are essential for genome integrity and chromosome stability. In many organisms including *T. brucei*, telomeres form a heterochromatic structure and suppress the expression of nearby genes, which is termed telomere position effect. *T. brucei*’s major surface antigen, VSG, is exclusively expressed from one of multiple VSG expression sites located immediately upstream of the telomere. Previously, we have shown that TBrAP1, a telomere protein, plays crucial roles in VSG regulation. In TBrAP1-depleted cells, nearly all subtelomeric VSGs were derepressed, and VSG switching occurs at a much higher frequency with VSG gene conversion being the predominant switching event. TBrAP1 also suppresses telomeric transcript (TERRA) and telomeric F-loop levels. All these TBrAP1 functions rely on its association with the telomere chromatin. We have recently shown that TBrAP1, unlike its homologues in yeast or vertebrates, has unique DNA binding activities that are required for its telomere association. However, it is still unknown how does TBrAP1 allows the active VSG to be fully expressed at a high level while silencing all other telomeric VSG genes. We have discovered that TBrAP1 has an RNA binding activity. Furthermore, structural analysis reveals an RRM motif in the TBrAP1 MyBlike domain, which is novel among all known RAP1 homologues. TBrAP1 has a much higher affinity to RNA than to DNA in *in vitro* EMSA analyses. In addition, the conserved F residues in the RRM motif are required for TBrAP1’s RNA binding activity. Moreover, *in vivo* characterization of point mutations in the TBrAP1 RRM motif that abolish the RNA binding provides more supporting evidence that the RNA binding activity of TBrAP1 is required for VSG monoallelic expression.

92 The location of *Trypanosoma cruzi* glycophosphoproteins in the plasma membrane is determined by their GPI anchor acceptor sequence

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At variance with mammals, trypanosomatids have a high percentage of proteins anchored by Glycosyl-Phosphatidyl-Inositol (GPI) into their plasma membrane. In *Trypanosoma cruzi*, GPI-anchored proteins such as trans-sialidase (TS) and mucins have a crucial role in the infection establishment. Despite being functionally related as an enzyme-substrate pair, these virulence factors are ordered in domains spatially separated from each other. We found mucins are located in detergent-resistant areas, whereas TSS are not. Currently, there is only scarce information concerning the signals that determine this arrangement on the plasma membrane. It is known that these two proteins have different GPI-lipidic composition, because of that we evaluated the involvement of GPI anchors in determining this membrane assortment. In order to investigate that, we constructed several recombinant genes fused to the Mucll-GPI or the TS-GPI signal coding sequence and cloned into a tetracycline-inducible expression vector. Using this regulated system allowed us to study both the final disposition in the plasma membrane as well as the intracellular trafficking of these recombinant proteins at different times post-induction. By analyzing the arrangement of these proteins on the cell surface, we observed a different protein domain pattern both in size and distribution. Besides, this correlated with the detection of proteins in detergent-resistant or non-detergent-resistant domains, depending on whether they carried the GPI anchor signal of Mucll or TS, respectively, highlighting the relevance of GPI anchors in the final destination of surface proteins. Concerning the intracellular trafficking, we also observed these proteins initially arranged in domains in the endoplasmatic reticulum (ER) endomembrane and detected them segregated in detergent-resistant regions in the ER-Golgi intermediate compartment (ERGIC). This early arrangement and sorting of proteins in different lipidic domains in the ER/ERGIC endomembranes could be essential to reach the high degree of order of the *T. cruzi* plasma membrane. Therefore, these results are relevant to understand how the paradoxical segregation of these virulence factors on the cell surface is achieved and reveal aspects of the organization of membrane proteins in *T. cruzi* early from its insertion into the cellular endomembranes.

93 A VSG epitope defined by calcium binding is associated with immunodominance in the host antibody repertoire

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The African trypanosome evades immune clearance through antigenic variation of its surface coat, repeatedly accessing a large genetic repertoire of divergent Variant Surface Glycoprotein (VSG) genes and “switching” to antigenically distinct versions. The VSG proteins have long been thought to function exclusively in antigenic variation through amino-acid variance on the protein surface. Recent evidence has challenged this notion, showing that the VSG coat can also be surface glycosylated and that such carbohydrate modifications can dramatically increase virulence and inhibit immune function. Further broadening the possible diversity in function of the VSG coat, we show here that the VSGs are not simply passive building blocks of coat divergence, but can also bind ligands. A 1.7Å resolution crystal structure of VSG2 reveals that it binds a metal. Mass spectrometric analysis reveals the presence of the ion and isothermal titration calorimetry confirms that VSG2 binds calcium with a Kd of 4.5µM. The crystal structure of VSG2 with mutations in the calcium coordinating residues (mutVSG2) presents disruption of calcium binding without structural changes relative to wtVSG2. The parasite mutants generate an antibody response which binds weakly to the mutVSG2 coat when compared to wtVSG2 antibody response which strongly binds wtVSG2 parasites. Assessment of the antibody repertoire reveals that wtVSG2 infection generates plasmablasts dominantly expressing two sets of antibodies with heavy chains defined by the VH10 family genes (set #1) or a specific J558 family gene (set #2) coupled with a restricted number of light chains. The set of antibodies composed by the VH10 heavy chain family segments is abrogated upon mutation of the residues involved in calcium coordination, indicating the calcium binding site to be fully expressed at a high level while silencing all other telomeric VSG genes. We have discovered that RAP1 functions rely on its association with the telomere chromatin. We have recently shown that RAP1 allows the active VSG to be fully expressed at a high level while silencing all other telomeric VSG genes. We have discovered that RAP1 has an RNA binding activity. Furthermore, structural analysis reveals an RRM motif in the RAP1 MyBlike domain, which is novel among all known RAP1 homologues. RAP1 has a much higher affinity to RNA than to DNA in *in vitro* EMSA analyses. In addition, the conserved F residues in the RRM motif are required for RAP1’s RNA binding activity. Moreover, *in vivo* characterization of point mutations in the RAP1 RRM motif that abolish the RNA binding provides more supporting evidence that the RNA binding activity of RAP1 is required for VSG monoallelic expression.

94 Development of a conditional protein degradation system to study essential gene function in the diarrheal parasite, *Cryptosporidium parvum*

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*Cryptosporidium parvum*, a protozoan parasite is an important veterinary and human pathogen, that causes diarrheal disease in ruminants (especially neonatal calves) and young children. There are currently no effective treatment and no vaccines available to treat or prevent *Cryptosporidium* infection (cryptosporidiosis) in animals and humans. This calls for the development of new drugs to reduce the morbidity and mortality associated with cryptosporidiosis and reduce parasite transmission. Therefore, it is critical to identify essential genes and study their biological functions in the parasite for the development of new and effective therapeutics. One of the challenges in studying parasite biology is that there are no genetic tools available to dissect function of essential genes in *Cryptosporidium* spp. We have overcome this challenge by developing the first conditional system to study essential gene function in *C. parvum*. We adapted the *Escherichia coli* dihydrofolate reductase degradation domain (DDD) and the stabilizing compound trimethoprim (TMP) to regulate protein levels in the parasite. We have tested our conditional system on the calcium-dependent protein kinase-1 (CDPK1), which is a leading drug target in *C. parvum*. Using a direct gene-knock-out strategy, we have established that *cdpk1* is essential for parasite survival. Our localization studies demonstrate that *cdpk1* is expressed during asexual proliferative stages of the parasite’s life cycle. Using clustered regularly interspersed short palindromic repeat (CRISPR)/Cas9 genetic editing, we generated stable transgenic CDPK1 parasites that express CDPK1 along with the DDD. We found that TMP can regulate CDPK1 levels in this stable transgenic parasite line, thus revealing the critical role of this kinase in parasite proliferation. Moreover, these transgenic parasites show TMP-mediated regulation of CDPK1 levels *in vitro*, and an increased sensitivity to kinase inhibitor upon conditional knockdown. This new conditional system would allow us to unravel functions of other essential Cryptosporidium genes in parasite biology, host-pathogen interactions and disease pathogenesis.
95 Use automated Spatially Targeted Optical Micro Proteomics (autoSTOMP) to explore the effector proteins near the parasitophorous vacuole membrane (PVM) of *Toxoplasma*.

Bocheng Yin, Sarah Ewald 1) University of Virginia.

The parasitophorous vacuole membrane (PVM) is the battlefield composed of secreted Toxoplasma components that recruit host nutrients and subvert an arsenal of host innate immune sensing components. Within PVM, the parasite proliferates while prevent severe inflammatory response causing host damage. This commensalism should maintain long enough to allow Toxoplasma entering a stage forming cyst that can survive to infect a next host. To identify components of the PVM involved in innate immune sensing, we perform a novel discovery proteomics technique called automated Spatially Targeted Optical Micro Proteomics (autoSTOMP) designed to identify the components of subcellular structures. In autoSTOMP, immunofluorescence microscopy identifies structures of interest (SOI) and tag the SOI proteins with biotin tag. Proteins tagged in this way are then precipitated and identified by mass spectrometry. We validated autoSTOMP can selectively identify the distinct proteomic profiles from PVM vicinity regions of Toxoplasma infected primary mouse bone marrow-derived dendritic cells (mMDCs). Next, we identify novel innate immune sensing components recruited to PVM in mMDCs to study the alternations that occurred at PVM interface by comparing host-intrinsic immune responses triggered by the activation of toll-like receptor ligands (the inflammasome) or interferon-γ (IFN-γ regulated GTPases). We found that the PVM components are distinct between stimulating conditions and generated a list of host response proteins. We validate a subset of these candidate proteins that serve in parasite clearance and host cell death and demonstrate that a balance between the survival of the parasite and host regulated near the PVM.

96 Untargeted metabolomic characterization of chronically infectious *Toxoplasma gondii* forms in a novel in vitro model identifies an unexpected mechanism of persistence.

Céline Christiansen1, Deborah Maus1, Jana Scho1, Florian Melerowicz1, Thobias Steinfeldt4, Matteo Murillo León1, Frank Seeber2, Michael Laue3, Martin Blume 1) Junior Research Group 2, Robert Koch-Institute, Berlin, Germany; 2) FG16: Mycotic and Parasite Agents and Mycobacteria, Robert Koch-Institute, Berlin, Germany; 3) Advanced Light and Electron Microscopy, Robert Koch-Institute, Berlin, Germany; 4) Institute of Virology, University of Freiburg, Freiburg, Germany.

Research on medically important persistor stages of many protozoan pathogens is limited by inadequate in vitro models. *T. gondii* resides life-long in brain and muscles of warm-blooded animals including an estimated third of humans globally. These chronic infections cannot be treated using available therapies. It also remains largely unknown how these parasites manage to persist and how their metabolism interacts on the host cell.

Here, we optimized the generation of mature *T. gondii* cysts in terminally differentiated human myotubes that act as natural host cells for the parasite. The resulting cysts resemble in vivo cysts in their resistance to drugs and heat as well as their ultrastructure, antigen expression and oral infectivity in mice.

To characterize the metabolome of *T. gondii* in vitro cysts we performed HILIC-UHPLC-MS-based untargeted metabolomics on these parasite forms for the first time. We show the presence of expected and unexpected metabolites in parasite cysts that include a number of imported host carnitines. Pharmacological modulation of host metabolism of carnitines and their subsequent import into the parasite reveals their implication in detoxification of antimicrobial fatty acids from the host.

97 Cellular barcoding reveals permissive host brain colonization by *Toxoplasma gondii*.

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Pathogenic infections and the diseases they cause are defined by the colonization of tissue niches. Host brain colonization is the clinically untreatable feature of persistent infection by the eukaryotic pathogen *Toxoplasma gondii*. The host blood-brain barrier is expected to physically restrict parasite colonization of this tissue niche and force the infection through a selection bottleneck, however technologies to test this have not been available. Here, we have invented a CRISPR-based method to barcode *Toxoplasma* parasites, and used libraries of barcoded parasites to define how the different phases of an infection shape the pathogen population structure. Unexpectedly, we have discovered that the murine host brain does not restrict parasite colonization, with the population structure predominantly shaped by a bottleneck in the acute phase of infection. Our finding supports an evolutionary strategy maximizing genetic diversity of parasite persisters within the intermediate host brain for subsequent transmission into the definitive feline host.

98 Single Cell Sequencing of *Plasmodium falciparum*.

Shiwei Liu, Adam Huckaby1, Audrey C. Brown1, Christopher C. Moore1, Ian Burbulis2, Michael McConnell1,3, Jennifer Guler1 1) University of Virginia; 2) Universidad San Sebastian; 3) Lieber Institute for Brain Development.

Antimalarial drugs are important weapons against the major human malaria parasite, *Plasmodium falciparum*. Their efficacy is mitigated by frequent emergence of resistant parasites. Copy number variations (CNVs, the amplification or deletion of a genomic region) are one of the major sources of genomic variation in *P. falciparum* that contribute to antimalarial resistance. Despite the importance of CNVs, their dynamics in evolving populations are not well understood. We hypothesize that CNVs constitutively arise in individual parasites, which poise the parasite population to rapidly respond to antimalarial exposure. However, such rare CNVs would not be detected when analyzing a population of parasites using next generation sequencing; their signal would be negated by the overwhelming number of parasites with normal copy number in that region of the genome. Single cell sequencing can be utilized to detect low-frequency CNVs within heterogeneous populations, but most single cell techniques are designed for mammalian cells. Here, we present a single cell sequencing pipeline for *Plasmodium falciparum*, which harbors a relatively small genome (23Mb) with an extreme base content (86.6% AT content). Through optimization of a quasi-linear amplification method, we reliably amplify early stage parasite genomes, better target the parasite genome over contaminants, reduce amplification bias and improve coverage breadth of the genome. By combining different CNV analysis tools (read-depth based tool: Ginkgo; split/discordant-read base tool: LUMPY), we can detect at least one of the 2 known CNVs (a 18kb CNV and a 80kb CNV) in 5 out of 25 single cell samples. We show the possibility of using cross-sample normalization to remove background noise caused by amplification bias and improve the accuracy of CNV detection at the single cell level.

Additionally, through stringent SNP filtering steps, like filtering out heterozygous SNP calls in haploid single parasite samples, we can detect SNPs in single cells. We validate a subset of these candidate proteins that serve in parasite clearance and cell death and demonstrate that a balance between the survival of the parasite and host regulated near the PVM.


Christian Reuter1, Fabian Imadhi,4 Laura Hauf,1 Tamara Finger2, Philipp Fey2, Heike Walles3, Antoine-Emmanuel Saliba4, Florian Groeber-Becker2, Markus Engstler1 1) Department of Cell and Developmental Biology, University of Wuerzburg, Germany; 2) Translational Center for Regenerative Therapies, Fraunhofer-Institute for Silicate Research, Germany; 3) Core Facility Tissue Engineering, University of Magdeburg, Germany; 4) Helmholtz Institute for RNA-based Infection Research, Helmholtz-Center for Infection Research, Germany.
Tsetse flies are the sole vector of African trypanosomes and the natural vector transmission involves the deposition of the parasites into the mammalian skin at the site of the fly’s bite. The knowledge about the early stages of infection is very limited because experimental approaches to host skin as first site of infection have been obstructed by the lack of appropriate animal models. Hence, primary human skin equivalents could provide a versatile model system for the investigation of vector-borne trypanosome infections in the skin. We have established a novel standardized human skin infection model with improved mechanical properties, which resembles native human skin in its histological architecture as well as in the development of distinctive physiological parameters. To simulate the natural infection process, we have successfully used tsetse flies for direct transmission of trypanosomes to the human skin equivalents. The injected parasites were deposited in the dermis in an unexpectedly complex manner, directly affecting their early distribution. The transmitted quiescent metacercarial trypanosomes were viable and showed a rapid transition to proliferative slender bloodstream forms. In addition, we have documented an intricate interaction of trypanosomes with collagen fibres and dermal fibroblasts. Tsetse flies have been successfully re-infected with skin-derived parasites, indicating completion of the mammalian life cycle of the parasites in the skin equivalents and full developmental competence of the parasites in the tsetse fly. To further strengthen our findings, we have embarked on a time-resolved single-cell transcriptome analysis of the skin-residing trypanosomes. Collectively, we established a new tool to get a better understanding of the role of the mammalian skin in African trypanosomiasis.

100 Bulk Segregant Approaches to Nutritional Genomics in Plasmodium falciparum in a Novel Plasmodium falciparum Genetic Cross Marko Kano1, Leila Ross1, Sacha Mok1, Tomas Yeo1, Melanie Shears2,4,5, Abhai Tripathi2, Felix Rozenberg3, Anne-Catrin Uhlemann3, Photini Sinnis2, David Fidock1,3 1) Department of Microbiology and Immunology, Columbia University Irving Medical Center, New York, NY; 2) Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, New York, NY; 4) Department of Laboratory Medicine, University of Washington Medical Center, Seattle, WA; 5) Center for Emerging and Re-emerging Infectious Diseases, Seattle, WA.

Chloroquine (CQ) was the former gold standard antimarial drug for Plasmodium falciparum from the mid-1940s – 1990s but was discontinued due to the prevalence of CQ-resistant (CQR) parasites; however, it is still in use for P. vivax. P. falciparum CQ resistance transporter (pfcrt) is known to be the primary driver of CQR in P. falciparum. Conversely, quinine (QN) is still in use to treat severe P. falciparum malaria and has been slow to develop resistance, mostly likely due to its complex, multifactorial mechanism of action and resistance that remains unsolved. To further our understanding of resistance to CQ, QN, and potentially related antimalarials, we conducted a P. falciparum genetic cross between Cam3.II (QNQR, CQR; pfcrtd2 pfmdr1184F) and NF54 (QNS, CQS; pfcrtdWT pfmdr1WT) using four F4G-NOD human liver-chimeric mice. Of the 163 progeny obtained, 56 were independent genetic recombinants. We performed ICSO assays and QTL analyses on 41, 34, and 44 synchronized progeny for CQ, mono-desethyl-CQ (the active metabolite of CQ), and QN, respectively. As expected, CQ and md-CQ QTL analyses identified an overlapping 13kb logarithm of odds (LOD) region on chromosome 7 where pfcrts lies. Interestingly, an additional overlapping 18kb region on chromosome 12 was identified. The peaks on chromosomes 7 and 12 were strongly additive, and accounted for 94-97% of the phenotypic variance for both drugs. We speculate that this chromosome 12 locus may augment the resistance phenotype driven by mutant pfcrts or compensate for its deleterious effects on growth. QTL analyses of QN also revealed LOD peaks on chromosomes 7 and 12 (1mb and 448kb), as well as some smaller peaks on other chromosomes. However, only the chromosome 12 peak appeared similar to that of CQ and md-CQ. These two peaks and an additional chromosome 13 peak collectively accounted for 84% of the phenotypic variance. We are currently obtaining more CQ- and QN-resistant progeny to refine the QTL loci, after which we will genetically validate the resistance gene candidates. Our study findings can help provide new insights into the mechanisms of action and resistance to CQ, QN, and related antimalarial compounds.

101 Identification of Multiple Determinants Associated with Chloroquine and Quinine Resistance in a Novel Plasmodium falciparum Genetic Cross Philip T. Xue1, Li Xue2, Samuel K. Sichang1, Mahmoud El-Gamil1,2, Richard W. LeMaster1,2,3, David E. Leach1,2,3 1) Department of Microbiology and Immunology, University of Alabama at Birmingham, Birmingham, AL; 2) Department of Pathology, University of Alabama at Birmingham, Birmingham, AL; 3) School of Public Health, University of Alabama at Birmingham, Birmingham, AL.

Plasmodium falciparum malaria and has been slow to develop resistance, mostly likely due to its complex, multifactorial mechanism of action and resistance that remains unsolved. To further our understanding of resistance to CQ, QN, and potentially related antimalarials, we conducted a P. falciparum genetic cross between Cam3.II (QNQR, CQR; pfcrtd2 pfmdr1184F) and NF54 (QNS, CQS; pfcrtdWT pfmdr1WT) using four F4G-NOD human liver-chimeric mice. Of the 163 progeny obtained, 56 were independent genetic recombinants. We performed ICSO assays and QTL analyses on 41, 34, and 44 synchronized progeny for CQ, mono-desethyl-CQ (the active metabolite of CQ), and QN, respectively. As expected, CQ and md-CQ QTL analyses identified an overlapping 13kb logarithm of odds (LOD) region on chromosome 7 where pfcrts lies. Interestingly, an additional overlapping 18kb region on chromosome 12 was identified. The peaks on chromosomes 7 and 12 were strongly additive, and accounted for 94-97% of the phenotypic variance for both drugs. We speculate that this chromosome 12 locus may augment the resistance phenotype driven by mutant pfcrts or compensate for its deleterious effects on growth. QTL analyses of QN also revealed LOD peaks on chromosomes 7 and 12 (1mb and 448kb), as well as some smaller peaks on other chromosomes. However, only the chromosome 12 peak appeared similar to that of CQ and md-CQ. These two peaks and an additional chromosome 13 peak collectively accounted for 84% of the phenotypic variance. We are currently obtaining more CQ- and QN-resistant progeny to refine the QTL loci, after which we will genetically validate the resistance gene candidates. Our study findings can help provide new insights into the mechanisms of action and resistance to CQ, QN, and related antimalarial compounds.

102 A single-cell RNAseq atlas of Schistosoma mansoni identifies a key regulator of blood feeding George Wendt1, Lu Zhaoh1, Rui Chen1, Chenxi Liu2, Anthony J. O’Donoghue2, Connor R. Caffrey2, Michael L. Reese1, James J. Collins III1 1) Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX; 2) Center for Discovery and Innovation in Parasitic Diseases, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA.

Schistosomes are intravascular flatworm parasites can dwell inside the host circulation for decades, where they feed on blood and lay eggs, causing the disease Schistosomiasis which infects over 240 million people. No vaccine available and emerging problem of the only drug increase the need for new therapeutic targets. As a metazoan comprised of multiple tissue types, understanding the schistosome’s biology on a molecular level during parasitism could suggest novel therapeutic strategies. Here we employ single-cell RNA sequencing to characterize 43,642 cells from the adult stage of schistosome including adult muscle, eggs, and 8 muscle clusters that possess unique expression patterns. Exploring the germline including germ stem cells (GSCs) and GSC progeny, we find no major sex- or maturation-dependent difference in early gametogenesis, but it’s sex-dependent in late female/male germ cells, and we observe a cellular lineage from stem cell to mature tissue both present in ovary and vitellaria maturation. We further uncover a lineage of somatic stem cells responsible for producing and maintaining the parasite’s gut – the primary tissue responsible for digestion of host blood. Finally, we show that a
homologue of hepatocyte nuclear factor 4 ([hnf4]) is expressed in this gut lineage and required for gut maintenance, blood feeding and inducing egg-associated pathology in vivo. Together, the data highlight the utility of this single-cell RNAseq atlas to understand schistosome biology and identify potential therapeutic interventions. Importantly, we also develop a web-based resource for accessing the data from our single cell atlas available at http://collinslab.org/schistocyte/, which will greatly benefit the scientific community.

**103 Establishment of quantitative RNAi-based forward genetics in Entamoeba histolytica and identification of genes required for growth** Akhila Bettadapour1, Katherine Ralston1 1) University of California, Davis; Davis, CA.

While *Entamoeba histolytica* remains a globally important pathogen, it is dramatically understudied. The genetic tractability of *E. histolytica* has historically been limited, which is largely due to its polyploidy, the large number of gene families, and high A/T content. To enable forward genetics, we constructed and validated the first *E. histolytica* RNAi knockdown mutant library. This library has genome-wide coverage, with the majority of genes represented by more than one unique fragment. The library design allows for Illumina deep sequencing analysis for quantitative identification of mutants that are enriched or depleted after selection. To precisely define and quantify gene fragments, we developed a novel analysis pipeline, where full-length DNA-fragments were inferred from read mapping results, rather than using more traditional read mapping coverage or CDS overlap counting strategies. We used a pilot version of this library to perform the first RNAi screen in *E. histolytica* and identified 12 slow growth mutants. Among genes targeted in slow growth mutants, many had annotated functions consistent with roles in cellular growth, and/or predicted roles in metabolic pathways. Some targeted genes were annotated as hypothetical or lacked annotated domains, supporting the power of forward genetics in uncovering functional information that cannot be gleaned from databases. Independently-generated mutants also exhibited slow growth phenotypes, showing that mutant phenotypes were reproducible. Finally, in addition to establishing forward genetics, we uncovered new details of the unusual *E. histolytica* RNAi pathway. These studies dramatically improve the tractability of *E. histolytica* and open up the possibility of applying genetics to improve understanding of this important human pathogen.

**104 High-throughput functionalization of the Toxoplasma gondii proteome** Tyler Smith1,2, Gabriella Lopez-Perez3, Emily Shortt1, Sebastien Lourido1,2 1) Whitehead Institute for Biomedical Research, Cambridge, MA; 2) Massachusetts Institute of Technology, Cambridge, MA; 3) University of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico.

Apicomplexans are some of nature’s most widespread parasites and include the causative agents of toxoplasmosis (*Toxoplasma gondii*), cryptosporidiosis (*Cryptosporidium* spp.), and malaria (*Plasmodium* spp.). These parasites have evolved an array of phylum-specific adaptations; however, most apicomplexan proteins have not been functionally characterized. Recently developed CRISPR-Cas9 screening platforms enable the high-throughput characterization of the *T. gondii* genome but lack temporal control and require follow-up studies to assign proteins to specific compartments and cellular pathways. We developed a high-throughput (HiT) CRISPR-mediated tagging vector to rapidly functionalize the C termini of target proteins with a synthetic sequence encoding protein tags or other regulatory elements. Utilizing the HiT vector, we tagged a library of 155 genes with the mini auxin-inducible degron (mAID) linked to a fluorophore and epitope tag. This enabled rapid and reversible knock-down of the targeted proteins. We assayed the function of each tagged mutant using pooled screens in the presence or absence of auxin. By incorporating distinct heterologous 3’ UTRs and tracking the abundance of guide RNAs over time in the untreated population we also identified potentially dosage-sensitive genes. After subcloning the populations we screened 1,160 arrayed clones by both lytic assay and microscopy via plate plating in the presence or absence of auxin. In addition to assigning protein localizations, we were able to place clones within 7 unique phenotype profiles, encompassing a diversity of parasite biology. This system extends the applications of genome-wide screens into complex cellular phenotypes, providing a new versatile platform for the dissection of apicomplexan cell biology.

**105 A SplitCas9 based phenotypic screen identifies two crucial genes involved in egress in Toxoplasma gondii** Wei Li1, Janessa Grech1, Johannes Felix Stortz2, Elena Jimenez-Ruiz1, Markus Meissner1 1) Ludwig-Maximilians-University (LMU) Munich, Munich; 2) University of Glasgow, Glasgow.

The apicomplexan parasite *Toxoplasma gondii* is an obligate intracellular parasite that can infect almost all warm-blooded animals, including humans. Exit from host cells is essential for the survival of the parasite and the dissemination of the infection. Egress is regarded as an active and highly regulated process. However, the details of this process of egress are still poorly understood.

During replication, the parasites establish an intravacuolar F-actin network that connects individual parasites and is essential for synchronous replication and material exchange between parasites. During egress, this network rapidly disintegrates before parasite motility and egress is activated. Importantly, stabilising this network, either by F-actin stabilising drugs or upon depletion of actin regulatory proteins, such as ADF (actin depolymerising factor) results in delayed or blocked egress, indicating a tight regulation of F-actin disassembly and activation of the motility machinery for egress. Importantly, similar effects can be seen in the case of merozoite egress of *Plasmodium falciparum*.

To identify novel factors involved in this regulation, we transfected a pooled gRNA library, which targets 320 genes predicted to be fitness-conferring according to a recent genome-wide in vitro screen, into a parasite line which expresses a regulatable Cas9 (split Cas9). In this case, the enzyme is expressed in two subunits fused to rapamycin binding domains, and addition of rapamycin reconstitutes its function. Disruption of the targeted genes allowed us to identify factors which participate in egress. These candidate genes seem to be involved in two different pathways that regulate these crucial processes.

**106A Entomological assessment of lymphatic filariasis transmission in Ghana** Dhikrullahi Shittu1, Yaw Afrane1, Simon Atta1, Mike Osei-Atweneboana2, Edward Tettevi2 1) University of Ghana, Accra, Ghana; 2) Council for Scientific and Industrial Research, Accra, Ghana.

Resurgence of lymphatic filariasis (LF) infection after several rounds of annual Mass Drug Administration (MDA) has been the main reason behind constant disease monitoring and surveillance to detect any possible hidden endemic foci. The MDA is designed to interrupt transmission by reducing *Wuchereria bancrofti* density in the blood of infected persons in a community to a threshold that local vectors may not be successful to pick up microfilariae (MF) during a blood meal. The current study was undertaken to entomologically assess LF transmission in endemic sites in Ghana where several rounds of MDA have been administered. Mosquitoes were collected in the study sites using the Pyrethrum spray collection (PSC), Human landing catches (HLC) both indoors and outdoors, and CDC light trap (LT) methods. The mosquitoes were identified morphologically to species level and later by Polymerase Chain Reaction (PCR) to distinguish sibling species. The presence of *Wuchereria bancrofti* was determined in the mosquitoes microscopically and further by PCR and Real Time-PCR. A total 700 mosquitoes were collected with 251 (35.9%), 99 (14.1%), 97 (13.9%) and 253 (36.1%) obtained from Anyakpo, Duase, Voggu Kpalogo and New Bakanta respectively. Microscopy did not identify any parasite. Polymerase Chain Reaction (PCR) detected one (1) pool being positive accounting for an infection rate of 0.43% each from Anyakpo and New Bakanta communities. Real time PCR (RT-PCR) detected *W. bancrofti* DNA signal in 17 (25.8%) of the pools. Of these, 13 (76.5%), 3 (17.7%) and 1 (5.9%) with an infection rate of 7.69%, 4.45% and 0.43% were from Anyakpo, Voggu Kpalogo and New Bakanta communities respectively. The study showed evidence of local transmission of LF in the mosquito and the mosquito species in the positive pools consisted of *An. gambiae* s.s, An. coluzzi and An. melas.
107A Environmental elasticity impacts parasite migration during transmission of malaria. Johanna Ripp1, Jessica Keher1, Xanthoula Smyrnakou1,2, Nathalie Tisch3, Carmen Ruiz de Almodovar3, Friedrich Frischknecht1 1) Heidelberg University Medical School; 2) Tübingen University Medical Center; 3) Heidelberg University.

Transmission of malaria-causing parasites to and by the mosquito rely on active parasite migration and constitute bottlenecks in the Plasmodium life cycle. Parasite adaption to the biochemically and physically different environments must hence be a key evolutionary driver for transmission efficiency. To probe how subtle but physiologically relevant changes in environmental elasticity impact parasite migration, we introduce 2D and 3D polyacrylamide gels to study ookinetes, the parasite forms emigrating from the mosquito blood meal and sporozoites, the forms transmitted to the vertebrate host. We show that ookinetes adapt their migratory path but not their speed to environmental elasticity and are motile for over 24 hours on soft substrates. In contrast, sporozoites evolved more short-lived rapid gliding motility for rapidly crossing the skin. Strikingly, sporozoites are highly sensitive to substrate elasticity possibly to avoid adhesion to soft endothelial cells on their long way to the liver. Hence the two migratory stages of Plasmodium evolved different strategies to overcome the physical challenges posed by the respective environments and barriers they encounter.

108A Just a spoonful of sugar helps the trypanosome hide: unraveling the antibody repertoires of O-glycosylated VSG3 and its sugar-less mutants. Anastasia Gikeka1,2, Gianna Triller1, Johan P. Zeelen3, Francisco Aresta-Branco1,3, Hamidreza Hashemi1, C. Erec Stebbins3, F. Nina Papavasiliou1 1) Division of Immune Diversity, German Cancer Research Centre (DKFZ), Heidelberg, Germany; 2) Faculty of Biosciences, Heidelberg University, Heidelberg, Germany; 3) Division of Structural Biology of Infection and Immunity, German Cancer Research Centre (DKFZ), Heidelberg, Germany.

Trypanosoma brucei is a flagellated unicellular parasite, that causes African sleeping sickness and is transmitted via tsetse flies. This pathogen is highly adapted for life in the host’s environment, since it evades immune responses by periodic changing of its dense coat of Variant Surface Glycoproteins. Our previous data regarding the structure of the variant VSG3 have revealed an O-linked carbohydrate (O-Glc) on the top of the NTD and that this sugar prevents immune recognition and pathogen clearance. We show here the presence of a second O-Glc on the NTD of VSG3 and characterize the antibody responses elicited by VSG3 and single-glycosylated or non-glycosylated mutants. We find that these differences in O-glycosylation lead to the specific VSGs being antigenically distinct, since their antibody repertoires are divergent. This suggests that the repertoires are elicited to very few immunodominant epitopes, enhancing immune evasion.

109A Small Molecule Based Disruption of an ApiAP2 Transcription Factor in the Human Malaria Parasite. Plasmodium falciparum. Timothy Russell1, Erandi K. de Silva2, Valerie Crowley1, Kathryn Shaw-Saliba3, Kyle J. McClean3, Gabrielle Josling1, Chrissie Florida Passa1, Gianni Panagiotou5, Jacquin Niles4, Marcelo Jacobs-Lorena3, Manuel Ullal1,6 1) Department of Biochemistry and Molecular Biology and Center for Malaria Research (CMaR), Pennsylvania State University; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University; 3) Department of Molecular Microbiology and Immunology, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health; 4) Department of Biological Engineering, Massachusetts Institute of Technology; 5) Leibniz Institute for Natural Products Research and Infection Biology, Hans Knöll Institute; 6) Department of Chemistry, Pennsylvania State University.

Precise timing of gene expression is critical for Plasmodium parasites to complete the major life cycle transitions that secure their proliferation. Key among the regulators of gene expression is the largest known family of sequence specific DNA binding transcription factors, the 27-member Apicomplexan AP2 (ApiAP2) family of proteins. ApiAP2 factors have no homology to the host due to their plant origins, making them attractive drug targets. Here, we report the first example of small molecule-based inhibition of an ApiAP2 protein. We identified potential inhibitors of DNA binding by screening thousands of publicly available compounds against the crystal structure of the AP2 DNA binding domain of PF3D7_1466040 in silico (Lindner et al. 2010). High scoring hits were determined to effectively kill P. falciparum in culture and to specifically inhibit DNA binding by the target protein in a gel shift assay. One compound (CID 3095349) was chosen as the lead for further experimentation. Remarkably, by injecting the lead compound into mosquitoes infected with P. berghei parasites, we found complete inhibition of salivary gland sporozoite formation. This result phenocopies the genetic deletion of the P. berghei orthologue of the target protein, AP2-Sp (Yuda et al. 2010). During asexual development of P. falciparum, treatment with the lead compound causes parasites to arrest at the trophozoite stage. Comparison of the P. falciparum asexual transcriptome at the onset of growth arrest to the genome occupancy of PF3D7_1466040 as measured by chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) revealed that a large fraction of transcripts affected by the lead compound are bound in the promoter region by AP2. This suggests that the target ApiAP2 protein is prevented from binding its cognate DNA loci and is therefore unable to activate transcription. We have generated several genetic approaches to conditionally deplete PF3D7_1466040 and measure the effect on parasite growth, and we hypothesize that the compound will phenocopy depletion of the target protein. Biophysical work is ongoing to characterize the interaction between the lead compound and the target AP2 domain. This work provides the first evidence that ApiAP2 proteins are a potential drug target against malaria parasites.

110A Elucidating the temporal events of Apolipoprotein L1-mediated African Trypanosome lysis. Sara Fresard1, 2, Joseph Verdi1,2,3, Jayne Raper1,2 1) Hunter College, New York, NY; 2) The Graduate Center at The City University of New York, New York, NY; 3) German Cancer Research Center, Heidelberg, Germany.

Trypanosoma brucei are extracellular parasites that are responsible for African sleeping sickness in humans, and nagana in livestock, which causes an agricultural and economic burden. Some species of primates are able to fight infection by trypanosomes due to an innate immunity factor called trypanosome lytic factor (TLF), which is a high-density lipoprotein, with a lytic, cation channel-forming component called Apolipoprotein L-1 (APOL1). The exact temporal mechanism of how APOL1 is able to lyse trypanosomes is disputed. The cation channel protein, APOL1, is taken into the trypanosome by receptor-mediated endocytosis, and once it reaches an acidic pH in the endosome, it inserts into the membrane. From here, the membrane, and associated APOL1, is recycled to the surface. APOL1 then encounters a neutral pH, allowing the cation channel to open. Other hypotheses include APOL1 trafficking to the mitochondria or lysosome from the endosome. Ultimately, there is cation flow that leads to osmotic imbalance within the parasite, resulting in cell lysis. This research examines the sodium, potassium, and calcium ion flux, mitochondrial and plasma membrane depolarization, and lysosomal pH maintenance using fluorescent biosensors and flow cytometry in order to determine the temporal events that result in APOL1-mediated lysis. This is crucial to investigate how some species of Trypanosoma brucei have become resistant to lysis by TLF, and also how TLF can safely be generated in livestock.

111A Toxoplasma gondii co-opts host repressor complexes to inhibit interferon induced necroptosis. Alex Rosenberg1, L. David Sibley1 1) Washington University School of Medicine in St. Louis.

During infection, Toxoplasma gondii translocate effector proteins directly into the infected host cells to subvert various immune signaling pathways. In this study, we utilized the APEX2 proximity labeling system to identify Toxoplasma gondii effector proteins that are secreted into and trafficked to the host cell nucleus. We identified all the known effectors and a novel secreted effector that antagonizes a cell death pathway to promote host cell survival. The novel secreted effector TgNSM localizes to the host cell nucleus and targets the activity of NCoR/SMRT repressor complex, which normally represses gene expression in a variety of pathways including immune responses. Mechanistically, TgNSM drives the increase in NCoR/SMRT levels and its subsequent transcriptional repression of interferon regulated genes (ISGs). TgNSM functions jointly with another T. gondii secreted effector Inhibitor of STAT1-dependent Transcription (TgIST), previously shown to down-modulate IFN-γ signaling. Although both type I (α/β) and type II (γ) IFNs provide protective roles they can also induce necroptosis that relies on RNA-responsive protein kinase PKR that induces the formation of a necrosome complex consisting of the receptor-interacting serine-
threonine kinase 1 (RIPK1) and receptor-interacting serine-threonine kinase 3 (RIPK3). The necroosome then activates the pro-necrotic protein mixed lineage kinase domain-like (MLKL) which drives the execution of necrotic cell death. TgNSM acts together with TgIST to block the IFN driven expression of PKR and MLKL, thus preventing host cell necrotic death and assuring survival of intracellular cysts. The mechanism of action of TgNSM represents a new model by which parasite counteracts host defenses, and a previously unappreciated role of NcOR/SRMT role in regulation of necroptosis.

112A Nanobodies Reveal Extensive Immunoaccessibility of the Trypanosome Coat

Alexander Hempelmann1, Monique Van Straaten1, Hamidreza Hashimi3, Nina Papasavliou3, C. Erc Cebesbi1, Laura Hartle2, Johan Zeelen1, Kathryn Perez4, Markus Engel1,2, Nicola Jones1 1) Division of Structural Biology of Infection and Immunity, German Cancer Research Center, Heidelberg, Germany; 2) Department of Cell and Developmental Biology, Theodor-Boveri-Institute, Biocenter, University of Würzburg, Würzburg, Germany; 3) Division of Immune Diversity, German Cancer Research Center, Heidelberg, Germany; 4) Protein Expression and Purification Core Facility, EMBL Heidelberg, Meyerhofstraße 1, Heidelberg, Germany.

The interaction of the trypanosome coat with the immune system represents the primary host-pathogen interaction surface in African Sleeping Sickness. The host develops neutralizing antibody responses, but the pathogen accesses an extensive genomic cache of antigenically distinct variant Surface Glycoprotein (VSG) genes that it expresses on the surface, avoiding clearance. In this study we develop nanobodies to probe the VSG surface. High-resolution crystal structures of VSG-nanobody complexes show that these camellid antibodies bind deep inside the coat. Coat-bound nanobodies can be detected with monoclonal antibodies, and full-length camellid antibodies as well, establishing that epitopes often considered buried in the densely packed surface are in fact accessible to immune surveillance. These results further our understanding of VSG-membrane dynamics as well as expand the epitope space of the VSGs, explaining the extensive surface divergence of these molecules in antigenic variation.

113A A conserved malaria parasite antigen Pb22 plays a critical role in male gametogenesis in Plasmodium berghei

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Transmission-blocking vaccines (TBVs) are potentially important tools for malaria eradication that interrupt parasite transmission. However, only a few TBV candidates are currently under development, highlighting an urgent demand to explore novel antigens for TBVs. In the present study, we selected a Plasmodium berghei protein (PBANKA_030590, named Pb22) using bioinformatics analysis and evaluated its potential transmission-blocking activity. This gene encodes a protein of 22 kDa (thus named Pb22) and is expressed in both asexual stages and gametocytes. BLAST search of it in GenBank identify its homologues in Plasmodium strains but not in other apicomplexans. Pb22 protein was localized in the cytosols of schizonts, as well as male and female gametocytes by an immunofluorescence assay (IFA). During gamete-to-ookinete development, Pb22 was localized on the plasma membranes of gametes, zygotes, and ookinetes. To elucidate the function of the gene, the gene was deleted (∆Pb22). ∆Pb22 exhibited a considerable influence on the parasite’s sexual stages as compared to wild type parasites, including a significant reduction in exflagellation (~89%) and ookinete numbers (~97%) when cultured with equal male gametocytes in vitro. A direct feeding assay showed an absolute blockade of ookinete and oocyst formation in midgut of the ∆Pb22 line. To confirm the influence of ∆Pb22 lines, we engineered Pb22-restoration (Pb22-RE) plasmds. Pb22-RE restored ookinete and oocyst numbers as well as infection prevalence (93.7%). Detailed analysis of male gametogenesis by IFA showed that 30% of the male gametocytes in the pb22-KO line failed to assemble the axonemes, whereas ~48.9% of the male gametocytes formed flagella but failed to egress from host erythrocyte. Cross-fertilization experiments further confirmed that ∆Pb22 male gametes are infertile whereas female gametes are fertile at wild type levels. To explore the transmission-blocking potential of Pb22, a full-length fragment of the predicted protein excluded signal peptide was expressed in bacteria and purified recombinant protein was used to immunize mice. Antisera against the recombinant protein elicted high IgG titers in mice. Transmission reducing ability (TBA) was measured in immune mice using culture assays. After infection with wild type P. berghei, a significant reduction in exflagellation (~65%) and ookinete numbers (~93.4%) were observed in in vitro culture assays. After blood meal for 3 days, a significant decrease in oocyst formation (93.5–99.6%) in the midguts as well as infection prevalence (83.3–93.3%) were observed. These data, together with gene conservation in Plasmodium, suggest that Pb22 could be a new TBV target candidate. Based on our results, further study of protein in human malaria parasites is warranted.

114A The Strategies of the Cloning Equilibrative Nucleoside Transporters (ENT's) family of Trichomonas vaginalis using Leishmania mexicana as a model organism

Manal Jamal Natto1, Tahani Abdulaziz ALSiari1, Harry de Koning1 1) Institute of Infection, Immunity and inflammation, Glasgow University, UK.

Trichomonas vaginalis (T. vaginalis) is a highly prevalent human urogenital protozoan parasite and the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection (STI) globally. Trichomoniasis can present as either acute or chronic, typically with a longer duration of infection in women than in men. Chronic infection of the reproductive tract leads to an increased risk of secondary infections, such as human immunodeficiency virus and other STIs. As with all protozoan parasites, T. vaginalis lacks de novo synthesis of purines. It is also unable to synthesise pyrimidine nucleotides, instead relying on salvage pathways as the main source of nutrients from the host. The salvage of nucleosides and nucleobases is believed to be dependent on the equilibrative nucleoside transporter (ENT) family, marking these proteins out as potential drug targets to disrupt the uptake of nutrients by the parasite. An ideal strategy to explore this further would be to express T. vaginalis ENT genes (TvENTs) by cloning them into a compatible heterologous system, where they can be studied individually to characterise the transport of nucleosides. Previous work has successfully sub-cloned TvENTs into a suitable expression vector for Trypanosoma brucei brucei, from which the adenosine transporter 1 has been deleted (TbAT1-KO). Nucleoside transport assays have identified high affinity of TvENT3 for cytidine and of TvENT6 for uridine. However, high background levels of uridine and purine nucleoside transport by trypanosomases confounded the results, prompting the search for a more reliable expression system. Fortunately, a Leishmania mexicana strain in which the native nucleoside transporter gene has been deleted by CRISPR/Cas9 provides one such system. The aim of this project is to develop this new system and evaluate its suitability for investigating the transport of nucleoside bases, potentially opening up a new and promising route to antitrichomonal drug discovery.

115A Fussing fission: defining apicomplexan cell division modes

Marc-Jan Gubbels1, Caroline Keroack2, Ciara Bauwens1, Srivney Danogoudriyam3, Hanna Worliczek4, Aditya Paul2, Brendan Elsworthy2, Clemens Engelberg1, Daniel Howe3, Isabelle Coppens5, Manoj Duraisingh2 1) Boston College, Chestnut Hill, MA; 2) Harvard T. H. Chan School of Public Health, Boston MA; 3) Gluck Equine Research Center, University of Kentucky, Lexington, KY; 4) University of Veterinary Medicine, Vienna, Austria; 5) Bloomberg School of Public Health, Johns Hopkins University, MD.

Cellular reproduction defines life, yet our textbook-level understanding of cell division is limited to a small number of model organisms centered around humans. The horizon on cell division variants is expanded here by advancing insights on the fascinating cell division modes found in the Apicomplexa, a key group of protozoan parasites. The Apicomplexa display remarkable variation in offspring number, whether karyokinesis follows each S/M-phase or not, and whether daughter cells bud in the cytoplasm or bud from the cortex. We find that the terminology used to describe the various manifestations of asexual apicomplexan cell division emphasizes either the number of offspring or site of budding, which are not directly comparable features and has led to confusion in the literature. Division modes have been primarily studied in two human pathogenic Apicomplexa, malaria-causing Plasmodium spp. and Toxoplasma gondii, a major cause of opportunistic infections. Plasmodium spp. divide asexually by schizogony, producing multiple daughters per division round through a cortical budding process, though several life-cycle nuclear amplifications are not followed by karyokinesis. T. gondii divides by endodyogeny producing two internally budding daughters per division round. Here we add to this diversity in replication mechanisms by considering the cattle parasite Babesia bigemina and the pig parasite Cystoisospora suis. B. bigemina produces two daughters per division round by a ‘binary fission’ mechanism whereas C. suis produces daughters through...
both endodyogeny and multiple internal budding known as endopolygeny. In addition, we provide new data from the causative agent of equine protozoal myelonecphalitis (EPM), Sarcocystis neurona, which also undergoes endopolygeny but differs from C. suis by maintaining a single multiploid nucleus. Overall, we operationally define two principally different division modes: internal budding found in cyst-forming Coccidia (comprising endodyogeny and two forms of endopolygeny) and external budding found in the other parasites studied (comprising the two forms of schizogony, binary fission and multiple fission). Progressive insights into the principles defining the molecular and cellular requirements for internal versus external budding, as well as variations encountered in sexual stages are discussed.

116A Metabolomic approaches to discovering resistance-resistant antimalarial drug targets Gabriel Range1, Kazutoyo Miura2, Elizabeth Winzeler3, David Fidock4,5, Manuel Llinás1 1) Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA; 2) Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD; 3) Department of Pediatrics, School of Medicine, University of California, San Diego, CA; 4) Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY; 5) Division of Infectious Diseases, Department of Medicine, Columbia University Medical Center, New York, NY.

Although enhanced efforts over the past two decades have significantly reduced the global malaria burden, the reduction in cases has stalled since 2014, indicating that current interventions are insufficient. One key challenge in reducing malaria impact is the rapid emergence of antimalarial drug resistance. Indeed, resistance against all modern antimalarials has emerged within 12 years of clinical introduction, including the current frontline treatments, artemisinin combination therapies. Thus, novel approaches to antimalarial development are required, with a deliberate intention to discover drug targets for which resistance development will be difficult. This study utilizes metabolomics to establish two approaches toward discovering resistance-resistant antimalarial targets.

First, while many Plasmodium falciparum mutations that enable antimalarial resistance have been well characterized, the impacts of these mutations on the overall metabolism of the parasite remain unexplored. By characterizing metabolic modifications triggered by resistance mutations, we will reveal novel vulnerabilities in antimalarial-resistant parasites. Additionally, understanding the metabolic adaptations required for resistance to frontline antimalarials will elucidate putative targets for partner drug development for use in directed multidrug therapies that lock parasites into a drug-sensitive state.

Second, the survival of P. falciparum is tightly intertwined with the nutrient sources of its various host environments. For the intraerythrocytic stages, nutrients in the serum produced by the host are essential for parasite symptom-causing asexual and transmissible gametocyte stages. However, the specific metabolites critical for parasite survival remain unclear. Capitalizing on the inherent variability between host serum in supporting P. falciparum asexual growth and gametocyte development and transmission, this study utilizes metabolomics to identify naturally variable serum factors and, thereby, targetable host processes that are integral for the malaria parasite. Crucially, developing resistance to host-targeted therapies would require the parasite to evolve a comprehensive circumvention of the host pathway, a potentially very large evolutionary hurdle that would render the development of resistance challenging.

117A Toxoplasma gondii AP2XII-2 contributes to proper progression through S-phase of the cell cycle Sandeep Srivastava1, Michael W. White2, William J. Sullivan1 1) Indiana University-Purdue University (IUPUI); 2) University of South Florida.

Toxoplasma gondii is a protozoan parasite that causes lifelong chronic infection that can reactivate in immunocompromised individuals. Upon infection, the replicative stage (tachyzoite) converts into a latent tissue cyst stage (bradyzoite). Like other apicomplexans, T. gondii possesses an extensive lineage of proteins called ApiAP2s that contain plant-like DNA-binding domains. The function of most ApiAP2s is unknown. We previously found that AP2XII-4 is a cell cycle-regulated ApiAP2 expressed only in dividing parasites as a putative transcriptional repressor. In this study, we purified proteins interacting with AP2XII-4, finding it to be a component of the recently characterized microchirida (MCOR) transcriptional repressor complex. We further analyzed AP2XII-2, another cell cycle-regulated factor that associates with AP2XII-4. We monitored parallel expression of AP2XII-4 and AP2XII-2 proteins in tachyzoites, detecting peak expression during S/M phase. Unlike AP2XII-4, which is dispensable in tachyzoites, loss of AP2XII-2 resulted in a slowed tachyzoite growth due to a delay in S-phase progression. We also found that AP2XII-2 depletion increased the frequency of bradyzoite differentiation in vitro. These results suggest that multiple AP2 factors collaborate to ensure proper cell cycle progression and tissue cyst formation in T. gondii.

118A In vitro evolution and population genomics identify intrinsically resistant Plasmodium falciparum isolates to Acyl Co-A Synthetase inhibitors Selena Bopp1,2, Charisse Pasaje3, Pamela Magistrado-Coxen1,2, Robert Summers1,2,4, Sumanta Dey3, Sebastian Smick3, Armiyaw Nasamu3, Allison Demasi1,2, Victoria Corey5, Maria Gomez-Loreno6, Virgina Franco6, Angela Early1,2, Amanda Lukens1,2, Danny Milner1,2, Francisco-Javier Gamó6, Elizabeth Winzeler5, Sarah Volkman1,2,7, Jacquin Niles3, Dyann Wirth1,2 1) Harvard T.H. Chan School of Public Health; 2) The Broad Institute; 3) Massachusetts Institute of Technology; 4) The Australian National University, Canberra; 5) University of California, San Diego; 6) Tres Cantos Medicines Development Campus; 7) College of Natural, Behavioral, and Health Sciences, Simons University.

The emergence and spread of drug resistance to current antimalarial therapeutics remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and prevent the development of drug-resistance. The Malaria Drug Accelerator (MalDA) consortium has adopted a chemogenomic approach to identify targets of the most promising compounds from chemically diverse libraries in Plasmodium falciparum in vitro cultures. This approach identified the acyl-CoA synthetase (ACS) enzyme family as new potential drug targets, where mutations in ACS10 and ACS11 were identified with selection of two unrelated compounds (MMV665924 and MMV019719). ACS11 and ACS10 are both members of the ACS gene family that consists of thirteen members. All Plasmodium species have four ACS orthologs (ACS9 to 12), which are predicted to perform classical ACS functions. However, nine paralogs have expanded and diverged from ACS9 in P. falciparum and P. reichenowi (ACS1a to 8). ACSs activate fatty acids (FA) scavenged from the host, which can then be used for protein modification, phospholipid biosynthesis, FA elongation, and beta-oxidation. We confirmed the role of the SNPs in ACS10 and ACS11 in resistance to the two compounds by introducing the mutations into the 3D7 parental line by the CRISPR/CAS9 system thereby phenocopying the drug resistance phenotype of the selected lines. We generated knock-down lines and confirmed that ACS10 is essential and the target of the drugs. Knock down of ACS11 on the other hand resulted in 80% protein reduction leading to a non-lethal growth defect and is most likely part of a drug resistance mechanism. ACS genes are highly conserved among Plasmodium species, suggesting a potential role in the overall metabolism of the parasite. By characterizing metabolic modifications triggered by resistance mutations, we will reveal potential targets for partner drug development for use in directed multidrug therapies that lock parasites into a drug-sensitive state.

119A In vivo imaging reveals that establishment of tissue reservoirs by Trypanosoma brucei is multi-factorial Mariana De Niz1, Daniela Bras1, Mafalda Pedro1, Claudio Franco1, Luisa Figueiredo1 1) Instituto de Medicina Molecular.

Trypanosoma brucei parasites have been defined as excellent swimmers in the body fluids of their hosts – both the insect vector, and various mammals. In their hosts, T. brucei parasites have the capacity to navigate in and out of the circulatory system, and to establish extravascular reservoirs in various tissues. Although both phenomena have been studied in ex vivo settings, we know very little about T. brucei dynamics in vivo. In our work, we used multiple imaging platforms to investigate cellular, physiological and biophysical factors that may contribute to the extensive colonization by T. brucei of specific tissues.
We began by defining *T. brucei* presence across organs throughout a full time course of infection using bioluminescence and intravitral microscopy. We identified various phenotypes across organs, namely those showing early parasite invasion (spleen, lymph nodes, liver), late parasite invasion (lungs, heart, kidneys), small reservoirs (brain), and large reservoirs (pancreas and adipose tissues). Our most striking findings were that a different dynamic of invasion exists across adipose tissue reservoirs (with white and brown adipose tissues differing the most), and that the pancreas is the largest *T. brucei* reservoir.

Next we studied host and parasite factors that could contribute to establishment of tissue reservoirs. First, we found that *T. brucei* large scale crossing of the blood vasculature is related to the day-light cycle. Second, we explored the roles of host vascular permeability, of vascular receptors using various mutant mice and blocking antibodies. We identified CD36, ICAM1, ICAM2, VCAM1 and various selectins to contribute to various extents, to the process of *T. brucei* extravasation. Abrogating reservoir establishment by blocking these host factors is detrimental to parasite survival (as measured in the blood periphery and individual organs loads), but still results in extensive pathology in the host. Third, we found that depending on the organ *T. brucei* traverses at different types of vessels. In the white adipose tissue parasites cross at vessels of small caliber, either venous or arterial origin. Finally, we identified specific parasite motility within the vasculature of different organs both under flow and upon blocking blood-flow. Overall, we conclude that establishment of tissue reservoirs is likely the result of a complex combination of circadian regulation, vascular receptors, and shear factors in blood flow.

**120A Elucidating the Functions of Polyamines for the Proliferation and Survival of Leishmania Parasites Sigrid Roberts1, Jasmine Perdeck1, Quintin Love1, Brandon Berioso1, Linh Le1, Nicole LoGiudice1, Yvette Leizorek1 1) Pacific University Oregon.**

Leishmaniasis is a neglected tropical disease affecting an estimated 12 million people annually. The absence of a vaccine and limited number of safe and effective treatment options calls for new drug development. Polyamines are metabolites that play central roles in the biology of all eukaryotes, and recent studies have highlighted their critical role in *Leishmania* parasites. The main polyamine biosynthetic enzymes in *Leishmania* are ornithine decarboxylase (ODC) and spermidine synthase (SPDSYN), which sequentially convert ornithine to the polyamines putrescine and spermidine. We have generated gene deletion mutants, *Δodc* and *Δspdsyn*, that show significantly reduced infectivity phenotypes in mice, validating this pathway as a potential therapeutic target. Our in vitro studies revealed putrescine-depleted Δodc parasites immediately ceased proliferation and died within two weeks. In contrast, putrescine-rich Δspdsyn mutants showed an intermediate growth phenotype and entered into a quiescent-like state with cell death occurring after six weeks. Together these studies led to the key finding that putrescine plays previously unrecognized yet vital roles for proliferation and survival. We are now in the process of elucidating the functions of putrescine for these key processes and to discern the molecular mechanisms leading to cell death in putrescine depleted cells and persistence in putrescine-rich cells. Preliminary studies suggest that putrescine is essential for replication and that putrescine depletion may lead to an apoptosis-like death. Our research aims to discover the functions of putrescine, offer novel insights into cellular processes essential for proliferation and survival, and may impact the development of therapeutic strategies against leishmaniasis.

**121A New Targets (Infiltrin) as a Vaccine Against Leishmaniasis Abdulaziz Alouffi1 1) King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia.**

Infiltrins (or pathogen-secreted host nucleus infiltrating proteins) are potential new targets for the development of more efficient vaccines against helminthic parasites. The archetypal infiltrin is SmIPSE (a.k.a. IPSE/alpha-1), a glycoprotein secreted by *Schistosoma mansoni* eggs, characterised by the simultaneous presence of a classical secretory and a nuclear localisation signal (CSS/NLS) signal. Within minutes following uptake by mammalian host cells, SmIPSE translocates to the nucleus and binds to DNA. This suggests that infiltrins, by acting e.g. as transcription factors, might play a central role in controlling the host-parasite relationship at the molecular level. Together with their secretory status, this role makes infiltrins interesting targets for vaccination.

Here, we analysed *Leishmania major* major protein sequence through use different bioinformatics tools to predict proteins that would show dual CSS/NLS signals. Secondly, we generated a series of truncated constructs fused with AcGFP1, which were transfected into mammalian cells. Nuclear localisation of fluorescence confirmed the existence of a single, monoparticulate C-terminal NLS in one of the *L. major* proteins. The predicted *L. major* candidate ‘IRRRKENKKRRN’ NLS motif, inserted into Tetra-EGFP, but not an Alanine NLS mutant, redirected the encoded ~100 kDa protein entirely to the nucleus. Use of an anti-his tag antibody shows that wild-type recombinant of the potential vaccine candidate, added exogenously to HEK293 cells, fully translocated to the nucleus, whereas the Alanine NLS mutant remained in the cytoplasm. Overall, the existence of an infiltrin in *L. major* suggests that infiltrins may represent a more general regulatory principle operating in parasite.

**122A Intestinal expression of mir-130b, mir-410b, and mir-98a in experimental canine echinococcosis by stem-loop RT-qPCR askhan faridi1,2, Ali Afgar2, Seyed Mohammad Mousavi2, Saeid Nasi2, Mohammad Ali Mohammad2, Mohammad Farajii abbas2, Majid Fasih Harand2 1) Student Research Committee, Kerman University of Medical Sciences, Kerman, Iran; 2) Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran.**

*Echinococcus granulosus* is a coelozoiccestode cestode small intestine as definitive hosts. A wide range of domestic and wild ungulates plays as intermediate hosts. Humans are dead-end and incidental intermediate hosts and become infected with the egg deposited from canid hosts that contaminated water and crude vegetables, where the larval stage causes cystic echinococcosis. Consider the nature and extent of molecular mechanisms involved in host-parasite interactions helps to answer some very basic questions in the biology of cestode parasites with significant implications in the management and control of cystic echinococcosis and propose a potent new molecular method of detection and biomarkers. The date about the miRNAs expression in the intestinal tissues of dogs infected with *E. granulosus* is scarce. In the present study expression of a selected miRNAs was evaluated in experimental canine echinococcosis.

miRNAs were isolated from 20 segments of small intestinal of two sibling dogs, one as control and the other was experimentally infected, through ingestion of sheep liver contain hydatid cyst with live protoscoleces. cDNA was specifically synthesized using an optimized stem-loop system design in the current study. Expression of four miRNAs (cfa-let7g, cfa-mir-98, cfa-mir-410, and cfa-mir-130b) was evaluated using RT-qPCR.

The results indicate a significant expression level difference between test and control dogs in cfa-mir-130b, cfa-mir-98, cfa-mir-410 (P≤0.05). However, there was no significant difference for cfa-let7g as it expressed in test and control dogs in a similar pattern. The most up-regulated miRNAs were cfa-mir-130b and cfa-mir-98. An increasing trend for cfa-let7g and, a declining trend for cfa-mir-98, cfa-mir-410, and cfa-mir-130b were found; towards the distal segments of the small intestine.

Our study revealed that cfa-mir-98, cfa-mir-410, and cfa-mir-130b are involved in the definitive host response in canine echinococcosis. The study provided new information on the molecular basis of interactions between *E. granulosus* and dog in terms of miRNA expression and showed that *E. granulosus* infection could increase the expression of some pro-inflammatory miRNAs at the cellular level in the definitive host. The current study paved a way to a more profound study in experimental infection of dogs infected with *E. granulosus*.

**123A Evaluation of Immunodiagnostic potential of schistosomula crude antigen (SCA) in Schistosoma mansoni infected human population Oyetunde Oyewumi1,2, Camila Amormino Corsini2, Rafaela Queiroz Grenfell2 1) University of Medical Sciences, Ondo, Nigeria; 2) Instituto Rene Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil.**
Early and efficient diagnosis can help to avert the morbidity associated with the infection. The aim of the study is to determine the potential of schistosomula crude antigen (SCA) as diagnostic biomarker for *Schistosoma mansoni*. The LE cercaria strain was transformed into schistosomula following a 3-h culturing in RPMI-1640 culture medium. The schistosomula were homogenized through sonication and centrifuged to obtain the SCA. The diagnostic potential of SCA was evaluated using ELISA and dot blots immunosassays on 30 *S. mansoni* infected and 30 non-infected humans’ sera samples. Dot blots immunosassay was further performed on protein eluted from 10-12 kDa immunoreactive band identified by Western blot analysis. The area under the ROC curve was 0.95 (AUC 0.95, CI 0.88-1.01, p < 0.0001). The sensitivity and specificity of SCA in diagnosing *S. mansoni* infected individuals using ELISA and dot blots assays were 96.67% and 86.67% respectively. The antibody titer produced against *S. mansoni* was significantly higher in *S. mansoni* infected individuals (OD=0.678±0.249) compared to the non-infected population (OD=0.235±0.136) (p < 0.0001). Our study showed outstanding diagnostic performance of SCA and its protein marker. Its preparation is easy and cheap, therefore, it can be adopted for immunodiagnosis of *S. mansoni* where the reference diagnostic antigens are not available.

124A Metabolic targets of miR-302/372/373/520 family in *Leishmania*-infected human THP-1 macrophages Juliane CR Ferrandes, Carla M Muxell, Lucile M Floeter-Winter
1) Biosciences Institute from University of São Paulo; 2) Tropical Medicine Institute from University of São Paulo Medical School.

*Leishmania* is an intracellular protozoan parasite that interfere with signaling mechanisms, immune function, and metabolism of host cells. During infection, the parasite switches murine macrophage L-arginine metabolism to synthesize polyamines, inhibiting nitric oxide production, which favors parasite replication. The mechanisms by which *Leishmania* controls the metabolism of human monocytes and macrophages remains elusive, since polarizing stimuli fail to induce significant nitric oxide synthase (NOS2) or arginase activity. Increase evidence points to central carbon metabolism as an important driver of human macrophage function. Additionally, posttranscriptional modulation of macrophage function by microRNAs (miRNAs) has been demonstrated during *Leishmania* infection.

We showed that human THP-1-derived macrophages infected with *L. amazonensis* had no statistically significant changes in expression of miRNAs related to polynucleine synthesis nor nitric oxide production. Quantification of macrophage miRNA profile showed upregulation of miR-302, miR-372, miR-373, and miR-520 during infection. This miRNAs family share the same seed sequence, region responsible for target-mRNA recognition. However, the validated target site for the murine homologous miR-294 is not conserved in human NOS2 mRNA. Bioinformatic analysis showed that glucose transporters are enriched among predicted targets of the human miR-302/372/373/520 family, revealing that the miRNAs GLUT1/SLC2A1, GLUT3/SLC2A3, and GLUT6/SLC2A6, previously observed in macrophages, are putative targets. We showed an increase in the GLUT3 transcript level in *Leishmania*-infected macrophages compared to non-infected control. Additionally, miRNAs of hexokinase 1, the first enzyme of the glycolytic pathway, and hypoxia inducible factor 1A (HIF1A), which induces glycolytic gene expression, are also putative targets. The aspartate-glutamate mitochondrial transporter SLC25A12 is a literature-validated target in viral infection.

Our results show that *Leishmania* infection of THP1-human macrophages upregulated miR-302/372/373/520 family that potentially targets transporters, enzymes and transcription factors related to glucose metabolism, but not L-arginine metabolism and transport. This result will be further exploited to validate the putative miRNA targets and to determine if glycolysis inhibition by post-transcriptional modulation affects *Leishmania* infectivity.

125A No vagina, one vagina, or multiple vaginae? An integrative study of the systematics of *Pseudaxine* and related genera

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The presence/absence and number of vaginae is a major characteristic for the systematics of the Monogenea. Three gastrocotyloid genera share similar morphology and anatomy but are distinguished by this character: *Pseudaxine* Parana & Perugia, 1890 has no vagina, *Allogastrocotyle* Nasir & Fuentes Zambrano, 1983 has two vaginae, and *Pseudaxinoides* Lebedev, 1968 has multiple vaginae. In the course of a study of *Pseudaxine trachuri* Parana & Perugia 1890, we found specimens with structures resembling “multiple vaginae”; we compared them with specimens without vaginae in terms of both morphology and molecular characteristiscs (COI barcode), and found that they belonged to the same species. We also investigated the male copulatory organ (MCO) of this species, the accuracy of the original description of which is known to be a matter of debate. We found that the genital atrium is armed with 12 hooks arranged as a single circle and a central hollow stylet which is probably involved in traumatic insemination. We redescribed *Pseudaxine trachuri* based on newly collected specimens from off the coast of Algeria and Museum specimens from off France. Specimens from the type-host, *Trachurus trachurus*, were found to be similar, for both molecular sequences and morphology, to those found on Boops boops. We can therefore confirm, for the first time with molecular evidence, that 8. boops is a host of this parasite. We consider that *Pseudaxinoides* was erected on the basis of an erroneous interpretation of structures which are not vaginae and, consequently, propose the transfer of most of its species to *Pseudaxine*, as *P. australis* (Lebedev, 1968), Bouguerche, Gey, Justine & Tazerouti, 2019, *P. bychowskyi* (Lebedev, 1977) Bouguerche, Gey, Justine & Tazerouti, 2019, *P. cabaleri* (Lebedev, 1977) Bouguerche, Gey, Justine & Tazerouti, 2019, *P. cariooaeosis* (Nasir & Fuentes-Zambrano, 1983) Bouguerche, Gey, Justine & Tazerouti, 2019, and *P. vietnamensis* (Lebedev, Parukhin & Roitman, 1970) Bouguerche, Gey, Justine & Tazerouti, 2019. We also propose *Allogastrocotyle dillonhargiros* Bouguerche, Gey, Justine & Tazerouti, 2019 for *Pseudaxine bivaginalis* Dillon & Hargis, 1965 to avoid a secondary homonymy.

126A Molecular detection and seasonal prevalence of Theileria annulata in blood samples of cattle collected from District Layyah in Punjab (Pakistan) Asia Parveen, Sherish Ashraf, Furhan Iqbal, Munir Akhtar
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The current study was designed to report the molecular detection and seasonal prevalence of *Theileria annulata* in cattle of cross breed, Holstein-Friesian and Sahiwal breed from District Layyah, Punjab, Pakistan and to document their phylogenetic origin and to report their association with studied epidemiological factors and complete blood count parameters, if any. A total of 844 blood samples (Cross = 244, Holstein-Friesian = 300, Sahiwal breed = 300) were collected during January 2017 till December 2018 from District Layyah on seasonal basis along with epidemiological data. Blood smear were prepared, stained with Giemsa and screened for *T. annulata* presence. Molecular detection was carried out by PCR amplification of Cytochrome b gene followed by DNA sequencing and phylogenetic analysis. Blood smear screening revealed 125/844 (15%) samples positive for *Theileria* species. PCR amplification of cytochrome b gene indicated an overall *T. annulata* prevalence of 21% (174/844). Highest parasite prevalence was in observed in autumn (53%) followed by winter (20%), summer (14%) and spring season (3%). Cross breed cattle were most susceptible to *T. annulata* (28%) followed by Sahiwal (19%) and Holstein-Friesian breed. Representative partial cytochrome b gene sequences of *T. annulata* were submitted to GenBank (Accession numbers MK0328444-46) and had phylogenetic similarities with sequences submitted from India, Iran, China, Turkey and Spain. Over all, it was observed that female animals and farm with water supply from pool, dogs living at farm and dogs having ticks load on them had significant association with *T. annulata* prevalence. Analysis of complete blood count data indicated that red blood cell, hemoglobin, mean cell hemoglobin, men corpuscular hemoglobin, lymphocyte (%), monocyte (%) and platelet count were significantly disturbed in *T. annulata* positive cattle of all three breeds. We recommend that this PCR protocol should be used for the detection of *Theileria annulata* in livestock for their proper diagnosis and treatment.

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127A Elevated *Plasmodium* sporozoite infection and multiple insecticide resistance in the principal malaria vectors *Anopheles funestus* and *Anopheles gambiae* in a forested locality close to the Yaoundé airport, Cameroon

*Nkemngo Francis Nongley*, 1 2 3 4

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**Background:** Reducing the burden of malaria requires better understanding of vector populations, particularly in forested regions where the incidence remains elevated. Here, we characterized malaria vectors in a locality near the Yaoundé international airport, Cameroon, including species composition, abundance, *Plasmodium* infection rate, insecticide resistance profiles and underlying resistance mechanisms.

**Methods:** Blood-fed adult mosquitoes resting indoors were aspirated from houses in April 2019 at Elende, a village located 2 km from the Yaoundé-Nsimalen airport. Female mosquitoes were forced to lay eggs to generate F adult progeny. Bioassays were performed to assess resistance profile to insecticides. The threshold of insecticide susceptibility was defined above 98% mortality rate and mortality rates below 90% were indicative of confirmed insecticide resistance. Furthermore, the molecular basis of resistance and *Plasmodium* infection rates were investigated.

**Results:** *Anopheles funestus* s.s. was most abundant species in Elende (85%) followed by *Anopheles gambiae* s.s. (15%) with both having a similar sporozoite rate. Both species exhibited high levels of resistance to pyrethroids (<40% mortality). *An. gambiae* s.s. was also resistant to DDT (9.9% mortality) and bendiocarb (54% mortality) while susceptible to organophosphate. *An. funestus* s.s. was resistant to deltamethrin (1% mortality), DDT (86% mortality) but susceptible to carbamates and organophosphates. The L119F-GSt2 resistance allele (8%) and G119S ace-1 resistance allele (15%) were detected in *An. funestus* s.s. and *An. gambiae* s.s., respectively. Furthermore, the high pyrethroid/DDT resistances in *An. gambiae* s.s. corresponded with an increase frequency of 1014F kdr allele (95%). Transcriptional profiling of candidate cytochrome P450 genes reveals the over-expression of CYP6P5, CYP6P9a and CYP6P9b.

**Conclusion:** The resistance to multiple insecticide classes observed in these vector populations alongside the high *Plasmodium* sporozoite rate highlights the challenges that vector control programs encounter in sustaining the regular benefits of contemporary insecticide-based control interventions in forested areas.

**128A A study on molecular detection, seasonal prevalence and phylogenetic evaluation of *Anaplasmata marginale* in blood samples of Cattle collected from District Layyah in Punjab (Pakistan) sehrish ashraf1, asia perveen1, furhan isba1, Mian Muhammad Awas 2, munir akta3, Sezayi Ozubek 3 1) Institute of Pure and Applied Biology Bahauddin Zakariya University Multan; 2) Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan 60800, Pakistan; 3) Fırat University, Veterinary Faculty, Department of Parasitology, 23119, Elazig, Turkey.

Anaplasma is known as yellow bag or yellow fever and it is a tick borne disease caused by obligate intercellular gram negative bacteria, *Anaplasmata marginale*. Present study is reporting seasonal prevalence, epidemiology and phylogeny of *Anaplasmata marginale* in three cattle breeds from District Layyah, Southern Punjab, Pakistan. A total of 844 blood samples (Cross = 300, Friesian = 244, Sahiwal breed = 300) from apparently healthy cattle on seasonal basis were collected along with epidemiological data. Polymerase chain reaction generated 265 base pair amplicon specific for Major surface protein-1b encoding gene of *Anaplasma marginale* in 8.6% (73/844) of enrolled cattle. Highest prevalence was observed during autumn (18.3%) followed by summer (9.7%) and winter season (7.1%). Friesian breed was most susceptible to *A. marginale* infection (13.1%) followed by Sahiwal (7.6%) and cross breed (6%). Representative amplified partial gene sequences of *A. marginale* were submitted to GeneBank (Accession numbers MK032842 and MK032843). Its phylogenetic analysis had shown similarities with sequences submitted by other countries. 37/844 (4.3%) Giemsa stained blood smears were found positive for *Anaplasmata sp*. Analysis of epidemiological factors revealed that female cattle and farm with water supply from pool, farms where other dairy animals and dogs were living with cattle and dogs having ticks load on them had significant association with *A. marginale* prevalence. It was observed that white blood cell, lymphocytes (%), Monocytes (%) heamatocrit, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were significantly disturbed in *A. marginale* positive than negative cattle. We recommend that this PCR protocol should be used for the detection of *A. marginale* in livestock for their proper diagnosis and treatment.

**Key words:** *Anaplasmata marginale*; Major surface protein-1b; PCR; Phylogenetic analysis; Cattle

129A The Spatial Organization of the *Plasmodium yoelii* DOZI/CITH/ALBA mRNP Complex During and After Translational Repression Kelly Rios1, Scott Lindner1 1) Department of Biochemistry and Molecular Biology, Buck Center for Research, and the Center for RNA Molecular Biology, Pennsylvania State University, University Park, PA, USA.

The transmission of *Plasmodium* parasites between mammalian hosts and mosquito vectors is integral to the parasite’s lifecycle and the spread of disease. The transmission stages represent major parasite population bottlenecks, and as such are ideal points of intervention for malaria eradication. A mechanism that may be exploited for such intervention is the translational repression of specific transcripts, in which genes are proactively transcribed and selectively blocked from translation. These mRNAs are stored in membraneless cytosolic messenger ribonucleoprotein (mRNP) granules until transmission occurs and translational repression is relieved. mRNA-binding proteins of the DOZI/CITH/ALBA complex localize to distinct, cytosolic mRNP granules and form a complex in female gametocytes to translational repress hundreds of mRNAs, including a specific transcription factor, pyap2-0, which is essential for parasite development in the mosquito. As many proteins found in this complex are expressed in both translationally active and repressive stages, we hypothesize that the spatial organization of this complex changes to accommodate changes in function. To test this, we are using complementary approaches of proximity-dependent protein interaction (Turbold) and super resolution imaging for single molecule fluorescence in situ hybridization (smFISH). We have C-terminally tagged two members of the DOZI/CITH/ALBA complex, PyDOZI and PyALBA4, that have been experimentally localized to the 5’ and 3’ ends of mRNAs, respectively. This strategy enables the determination of spatial interactions, from which changes in the subcellular organization of proteins within this complex may be inferred, and whether the extension/compaction of mRNA correlates with translational status in gametocytes and early mosquito stages. Additionally, we are using super resolution single-molecule RNA-FISH in pre- and post-transmission stage parasites to determine if the spatial compaction/expansion of a translationally repressed transcript, pyap2-0, correlates with translational status. These two complementary approaches, TurboID and smFISH, will allow us to monitor the spatial organization of the proteins and mRNAs in this complex at translationally repressive and translationally permissive stages of the parasite lifecycle. From this, we will better appreciate how translational repression is mediated in gametocytes, and if repressed mRNAs are similarly compacted in protein and mRNAs in this complex at translationally repressive and translationally permissive stages of the parasite lifecycle. From this, we will better appreciate how translational repression is mediated in gametocytes, and if repressed mRNAs are similarly compacted in transmission granules as they are in human stress granules.

130A *Trypanosoma brucei* TIF3 is a novel telomere protein that is essential for cell viability and affects VSG switching *Brittney Schnur1, Marija Afrin1, Bibi Li1 1) Cleveland State University, Cleveland, OH, USA.

*Trypanosoma brucei* causes sleeping sickness in humans and nagana in cattle. While proliferating inside its mammalian host, T. brucei stays in extracellular space and elicits strong host immune response. However, T. brucei regularly switches its major surface antigen, VSG, and effectively evades elimination by the host immune system. VSGs are expressed exclusively from subtelomeric expression sites (ESs) in a strictly monoallelic manner. VSG is the last gene in any ES and is located immediately upstream of the telomeric repeats. Telomeres are nucleoprotein complexes at chromosome ends and are essential for regular chromosomal stability. TIF3 is a telomere protein that is essential for cell viability and affects VSG switching.

**Key words** Cytochrome b gene; PCR; *T. brucei* annulata; Phylogenetic analysis; Cattle.
genome integrity and chromosome stability. VSG switching can occur through DNA recombination or by changing of transcriptional states of different ESs. We have previously identified TbTRF as the duplex telomere DNA binding factor and both TbRAP1 and TbTIF2 as TbTRF-interacting factors. All these telomere proteins are essential for T. brucei viability. They all suppress DNA recombination-mediated VSG switching, although the underlying mechanisms may vary. Additionally, TbRAP1 is essential for VSG silencing. Now we have identified another TbTRF-interacting factor in a yeast 2-hybrid screen, which we named TbTIF3 (TbTRF Interacting Factor 3). We found that depletion of TbTIF3 causes an acute cell growth arrest, indicating that TbTIF3 is essential for T. brucei viability. Additionally, a transient depletion of TbTIF3 leads to an increase in VSG switching frequency, suggesting that TbTIF3 may function in the same pathway as TbTRF and TbTIF2.

131A Rap1b activation and ERK phosphorylation during cAMP/Epac-mediated invasion by Trypanosoma cruzi Gabriel Ferrell1, Daniel A. Musikant2, Martin M. Edereira1,2,3 1) CONICET-Universidad de Buenos Aires, Instituto de Quimica Biologica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires; 2) Departamento de Quimica Biologica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires; 3) Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh, Pittsburgh.

Cyclic AMP has been shown to play critical roles during host cell invasion by T. cruzi. Ca2+ release from cellular compartments, such as the endoplasmic reticulum, is accompanied by an elevation of intracellular cAMP levels and it has been shown that cAMP is able to potentiate the Ca2+-dependent exocytosis of lysosomes during host cell invasion. We previously demonstrated that Epac1-mediated signaling represents the main mechanism for cAMP-mediated host cell invasion. Furthermore, Epac1 has been involved in PI3K/Akt and MEK/ERK pathways, and members of these pathways, including Rap1, were localized at late endosomes/lysosomes. In this work, we investigated the involvement of two downstream effectors, Rap1b and ERK, in Epac-mediated invasion. Active GTP-bound Rap1 was detected in lysates from infected cells by pull-down experiments through specific protein interaction with a GST-RalGDS Rap1-binding domain. In line with these results, invasion significantly increased in cells transfected with the constitutively active form of Rap1b (G12V) when compared with the DMSO control and a dominant negative mutant of Rap1b (N17). Regarding ERK participation, we first showed that trypomastigotes induced ERK phosphorylation. In addition, treatment with PD98059, an inhibitor of MEK1/2, suppressed ERK phosphorylation and induced a significant decrease in T. cruzi invasion. Interestingly, co-inhibition of Epac1 and ERK phosphorylation showed no additive or synergistic effects, suggesting that ERK is a downstream effector of cAMP/Epac-mediated host cell invasion.

132A Generation of Novel Plasmodium falciparum NF135 and NF54 Lines Expressing Fluorescent Reporter Proteins Under the Control of Strong and Constitutive Promoters Shinya Miyazaki1, Annie S.P. Yang2, Fiona J. A. Geurten1, Catherin Marin-Mogollon1, Yukiko Miyazaki1, Takashi Imai1,3, Surendra Kumar Koli1, Jai Ramesar1, Severine Chevalley-Maurel1, Ahmed M. Salman4, Geert-Jan A. van Gemert2, Youri M. van Waardenburg2, Blandine Franke-Fayard1, Adrian V. S. Hili4, Robert W. Sauerwein2,5, Chris J. Janse1, Shahid M. Khan1 1) Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands; 2) Department of Medical Microbiology, Radboud University Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, Netherlands; 3) Department of Infectious Diseases and Host Defense, Gunma University Graduate School of Medicine, Maebashi, Japan; 4) Nuffield Department of Medicine, The Jenner Institute, University of Oxford, Oxford, United Kingdom; 5) TropIQ Health Sciences, Nijmegen, Netherlands.

Transgenic reporter lines of malaria parasites that express fluorescent or luminescent proteins are valuable tools for drug and vaccine screening assays as well as to interrogate parasite gene function. Different Plasmodium falciparum (Pf) reporter lines exist, however nearly all have been created in the African NF54/3D7 laboratory strain. Here we describe the generation of novel reporter lines, using CRISPR/Cas9 gene modification, both in the standard Pf NF54 background and in a recently described Cambodian P. falciparum NF135.C10 line. Sporozoites of this line show more effective hepatocyte invasion and enhanced liver merozoite development compared to Pf NF54. We first generated Pf NF54 reporter parasites to analyze two novel promoters for constitutive and high expression of mCherry-luciferase and GFP in blood and mosquito stages. The promoter sequences were selected based on available transcriptome data and are derived from two housekeeping genes, translation initiation factors (SUI1, putative (sui1_PF3D7_1243600) and 40S ribosomal protein S30 (40s_PF3D7_0219200). We then generated and characterized reporter lines in the Pf NF135.C10 line which express GFP driven by the sui1 and 40s promoters as well as by the previously used ef1a promoter (GFP@ef1a, GFP@sui1, GFP@40s). The GFP@40s reporter line showed strongest GFP expression in liver stages as compared to the other two lines. The strength of reporter expression by the 40s promoter throughout the complete life cycle, including liver stages, makes transgenic lines expressing reporters by the 40s promoter valuable novel tools for analyses of P. falciparum.
135A Ribosome heterogeneity and potential functional differences in the specialized ribosomes of Plasmodium spp. James McGee1, Scott Lindner1 1) Department of Biochemistry and Molecular Biology, Hück Center for Malaria Research, Pennsylvania State University.

Eukaryotes commonly have hundreds of copies of genes coding for ribosomal RNA (rRNA) in their genomes, and often only have slight sequence differences between them. Plasmodium species differ from other eukaryotes in that they have far fewer (4-5) rRNA gene copies in their genome. Moreover, the expression of specific rRNA genes is controlled in a stage-specific manner throughout their life cycle, with two or three clusters dedicated to Aseua1-type (A-type) rRNA expression and two clusters dedicated to Sporozoite-type (S-type) rRNA expression. Previous work has demonstrated that each S-type rRNA gene is critical, but not essential, for mosquito stage development. Recently, the re-discovery of specialized ribosomes in eukaryotes has prompted studies to understand the importance of ribosome heterogeneity and the potential for specialized function. This has been strengthened further by the discoveries of functional and specialized rRNA expansion segments, ribosomal proteins (RPs), RP paralogs, ribosome associated proteins (RAPs), and post-transcriptional and post-translational modifications to components of the ribosome complex. Here, we describe key differences in Plasmodium ribosome types and focus on different expansion segments in their rRNA sequences and comparisons of their predicted secondary structures. We also describe the use of CRISPR-RgR for simultaneous, multi-locus gene editing to investigate the essentiality of specific rRNA features and demonstrate the application of the Ribolace method to isolate and study Plasmodium ribosomes.

136A Dissecting the regulatory role of an enriched DNA sequence motif found upstream of Plasmodium falciparum gametocyte-associated genes Riete van Bijl1, Timothy Russell1, Manuel Linares1,2 1) Department of Biochemistry & Molecular Biology and the Hück Center for Malaria Research, Pennsylvania State University, University Park, PA; 2) 2Department of Chemistry, Pennsylvania State University, University Park, PA.

The formation of mature Plasmodium gametocytes is a critical stage in ensuring malaria transmission. In Plasmodium falciparum parasites, gametocyto genesis is a considerably longer process (~14 days) than almost all other Plasmodium species (2-3 days). Following expression of PfAP2-G, the well-established master regulator of gametocyte commitment, there are additional regulatory mechanisms guiding further gametocyte development and maturation. We have identified an enriched DNA sequence motif (AGACA) upstream of genes whose mRNA abundance peak in the intermediate to late stage gametocytes. Although this motif has been associated with P. falciparum sexual development by several transcriptome studies there has been no link to a specific trans-acting factor associated with this motif. Our preliminary studies support that AGACA is a gametocyte-specific motif, since it is bound in nuclear lysate extracted from P. falciparum gametocytes but not in asexual parasites. DNA pull-down assays using biotinylated oligomers containing the AGACA motif and gametocyte lysate have consistently enriched for an uncharacterized protein with orthologues in most Plasmodium species that contains a CCCH zinc finger domain. The gene encoding for this zinc finger protein was not essential for asexual development in P. berghei (RMgm-1902) and dispensable by mutation through piggyBac transposon mutagenesis in P. falciparum. We are currently testing whether this zinc-finger protein is able to bind AGACA in vitro and in vivo. Using a genetic knock out parasite line we are also investigating the role of this protein in the regulation of transcription and/or translation in gametocyte development. 

137A Kinetoplast RNA Editing Helicase 1 plays a distinct role in RNA editing profile of mitochondrial transcripts in Trypanosoma brucei Amartya Mishra1, Ashutosh P. Dubey1, Brianna L. Tyle1, Laurie K. Read1 1) Department of Microbiology and Immunology, State University of New York at Buffalo

In trypanosomes, most mitochondrial encoded mRNAs require post-transcriptional uridine (U) insertion/deletion (U-indel) editing, which is directed by trans-acting gRNAs and mediated by a holoenzyme comprised of the catalytic RECC, the REH2C helicase complex, and non-catalytic RNA editing substrate binding complex (RESC). Another ATP-dependent RNA helicase, Kinetoplast RNA Editing Helicase 1 (KREH1), interacts transiently with RECC and RESC. Previous evidence suggested a role for KREH1 gRNA removal during editing progression. Here, we show that a KREH1 null mutant (KO) in procyclic form T. brucei exhibits a modest decrease in a subset of edited mRNAs compared to wild type (WT), in particular ATPase (A6) mRNA. High throughput sequencing followed by TREAT analysis of A6 mRNA in KREH1 KO cells revealed no evidence for KREH1 function in gRNA removal. Rather, we identified multiple sequences in the KREH1 KO with a long stretch of pre-edited sequence followed by one or two modified editing sites outside of the first gRNA. These data suggest a role of KREH1 in modulation of RNA structure. Overexpression (OE) of WT KREH1 from an ectopic locus confers a significant growth defect and leads to a 75-90% decrease in fully edited versions of most pan-edited mRNAs while pre-edited mRNAs were unaffected. Moderately edited mRNAs were largely unaffected. Together, these data suggest that 3’ to 5’ editing progression is impaired in KREH1 KO. To define the role of KREH1 ATP-binding, we overexpressed KREH1 mutated in the conserved ATP-dependent binding motif. Mutant OE leads to cell death, indicating a dominant negative (DN) phenotype. This is accompanied by a dramatic decrease in almost all edited mRNAs with concomitant accumulation of pre-edited transcripts, including 2 of 3 moderately edited mRNAs. Thus, unlike WT KREH1, the DN mutant impairs initiation of editing. WT and DN KREH1 bind RESC and RECC to a similar extent. However, the OE of DN KREH1 alters RESC homeostasis by interfering with the interaction between its GRBC module and a subset of additional RESC proteins, including some previously shown to be essential for editing initiation in vivo. Together, these data are consistent with a model in which GRBC and RECC assemble normally on mRNA, while KREH1-mediated ATP hydrolysis is necessary for subsequent assembly of a functional RESC, capable of promoting editing initiation. Thus, KREH1 is important for both initiation and progression of U-indel editing.

138A The calcium-responsive proteome of an apicomplexan parasite Alice Herneisen1, Marc-Jan Gubbels2, Sebastian Lourido1 1) Whitehead Institute and Massachusetts Institute of Technology; 2) Boston College.

As obligate intracellular pathogens, apicomplexans such as T. gondii are exquisitely tuned to transduce environmental signals into programs of motility, replication, and quiescence responsible for parasite spread and pathogenesis. Second messengers, like calcium, mediate this signal transduction. Although components of second messenger signaling pathways are conserved among eukaryotes, the cognate parasite proteins remain unknown and unidentified by sequence searching due their evolutionary divergence. To address these challenges, we have leveraged global, quantitative mass spectrometry to identify second messenger signaling components de novo in T. gondii. Thermal proteome profiling systematically identified calcium-responsive proteins on the basis of their biochemical thermal stability. In addition to conserved calcium-responsive proteins and an expanded parasite family of calcium-dependent protein kinases, many of the proteins exhibiting calcium-responsive behavior are parasite-specific proteins lacking characterization. We also identified proteins that portend unexplored mechanisms of crosstalk between pathways. To elucidate the layers of calcium regulation in parasites, we characterized calmodulin-responsive proteins using orthogonal proteomic methods combined with conditional genetics. Calmodulin coordinates the integrity of a motor at the parasite apex required for motility and invasion. Together, our experiments expand the known calcium-responsive proteome of divergent human parasites and are the first steps to systematically reconstruct the targets of second messenger signaling de novo.
139A Interaction of Signaling Lymphocytic Activation Molecule Family 1 (SLAMF1) receptor with Trypanosoma cruzi is strain-dependent and affects NADPH oxidase expression and activity in macrophages. Cristina Poveda1, Alfonso Herreros-Cabelló1, Francisco Callejas-Hernández1, Jesús Osuna-Pérez1, María C. Maza1, Carlos Chilión-Marinas1, Jossela Calderón1, Konstantinos Stamatakis1, Manuel Fresno1,2, Núria Gironés1,2 1) Centro de Biología Molecular Severo Ochoa (CSIC-UMA), Madrid, Spain; 2) Instituto Sanitario de Investigación Princesa, Madrid, Spain.

Chagas disease, caused by the parasite Trypanosoma cruzi (T. cruzi), is characterized by an acute phase with low mortality, and after many years without any sign of disease, patients develop a symptomatic chronic phase, characterized by cardiomyopathy and/or digestive mega syndromes. These differences have been attributed to the high genetic variability of this parasite and at least 6 different Discrete Units (DTUs) have been established. A relation between DTUs and clinical symptoms has been hypothesized, although the immunopathology of the disease is still not completely understood.

The receptor Signaling Lymphocytic-Activation Molecule Family 1 (SLAMF1 or CD150) controls susceptibility to infection by the lethal T. cruzi Y strain. SLAMF1 is required for macrophage phagocytosis and phagosome localization where it enhances the activation of NADPH oxidase (NOX2). To elucidate if the genetic diversity of the parasite was related with disease susceptibility, we analyzed the role of SLAMF1 studying the behavior in vitro of six strains representative of each DTU (Dm28c, Y, M6421 c6, 10R26, Bug2148 c1 and VFRA c1) in macrophages isolated from BALB/c and Slamt1-/- mice. The interaction of SLAMF1 receptor with T. cruzi was evidenced by fluorescence microscopy, flow cytometry and quantitative PCR.

Different strains interact, internalize and replicate in BALB/c macrophages with varied efficiency and all of them, except VFRA, showed a decrease in parasite load in infected macrophages in Slamt1-/- compared to BALB/c. The different observed patterns of gene expression clearly differentiated between the three phases of macrophage infection, with relevant distinctions between Slamt1-/- and BALB/c in some genes as Tnf, Ilg1, Il1b and Ifg6. Also, we found important differences regarding NOX2 expression and ROS production by macrophages: both decreased in BALB/c compared to Slamt1-/- with all the strains, except VFRA, in which we found the opposite behavior.

Thus, our results indicate that the SLAMF1 receptor interacts with T. cruzi affecting parasite replication and ROS production in macrophages in a parasite strain-dependent manner. Furthermore, they suggest that VFRA strain may not strictly require SLAMF1 for interaction and internalization as the other strains did. However, VFRA was able to trigger more efficiently ROS production than the others in a Slamt1-/-dependent manner, indicating a functional interaction with SLAMF1, although of the opposite sign.

140A Role of Plasmodium falciparum Kelch 13 Protein Mutations in P. falciparum Populations from Northeastern Myanmar in Mediating Artemisinin Resistance Faiza Siddiqui1, Rachasak Boonhok1, Mynthia Cabrera2, Huguette Gaelle Ngassa Mbenda1, Meilian Wang3, Hui Min1, Xiaoying Liang1, Junling Qin1, Xiaotong Zhen3, Jun Miao1, Yaming Cao3, Liwang Cui1 1) Department of Internal Medicine, University of South Florida, Tampa, Florida, USA; 2) Department of Biochemistry & Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania, USA; 3) College of Basic Medical Sciences, China Medical University, Shenyang, Liaoning, China.

Mutations in the Plasmodium falciparum Kelch 13 (PK13) protein are associated with artemisinin resistance. PK13 is essential for asexual erythrocytic development, but its function is not known. We tagged the PK13 protein with green fluorescent protein in P. falciparum to study its expression and localization in asexual and sexual stages. We used a new antibody against PK13 to show that the PK13 protein is expressed ubiquitously in both asexual erythrocytic stages and gametocytes and is localized in punctate structures, partially overlapping an endoplasmic reticulum marker. We introduced into the 3D7 strain four PK13 mutations (F446I, N458Y, C469Y, and F495L) identified in parasites from the China-Myanmar border area and characterized the in vitro artemisinin response phenotypes of the mutants. We found that all the parasites with the introduced PK13 mutations showed higher survival rates in the ring-stage survival assay (RSA) than the wild-type (WT) control, but only parasites with N458Y displayed a significantly higher RSA value (26.3%) than the WT control. After these PK13 mutations were reverted back to the WT in field parasite isolates, all revertant parasites except those with the C469Y mutation showed significantly lower RSA values than their respective parental isolates. Although the 3D7 parasites with introduced F446I, the predominant PK13 mutation in northern Myanmar, did not show significantly higher RSA values than the WT, they had prolonged ring-stage development and showed very little fitness cost in in vitro culture competition assays. In comparison, parasites with the N458Y mutations also had a prolonged ring stage and showed upregulated resistance pathways in response to artemisinin, but this mutation produced a significant fitness cost, potentially leading to their lower prevalence in the Greater Mekong subregion.

141A Investigation of sequence-specific transcription factors with similar DNA binding specificities in the human malaria parasite Plasmodium falciparum Victoria A Bonnell1, Gabrelle A Josling1, Yuning Zhang2, John Horton2, Raluca Gordan2, Manuel Llinas1 1) The Pennsylvania State University; 2) Duke University.

About 90% of the protein-coding genes from the Plasmodium falciparum genome are transcribed in a periodic fashion with the peak transcript abundance occurring just before the protein is required during the 48-hour intraerythrocytic developmental cycle. However, despite having a tightly coordinated transcriptional network, the P. falciparum genome codes very few canonical eukaryotic transcription factors (TFs). This work aims to understand the biological function and binding specificity of a subset of Apicomplexan APETALA 2 (ApiAP2) proteins, which are the largest-known family of sequence-specific TFs in Apicomplexan parasites described to date. While many members of the P. falciparum ApiAP2 family recognize similar DNA sequence motifs in vitro, several AP2 DNA binding domains recognize similar DNA sequence motifs. In higher eukaryotes, TFs with similar binding preferences (i.e. paralogous TFs) can carry out divergent regulatory functions in a given cell type, work synergistically or antagonistically, perform similar functions in different cell types, or can be functionally redundant. Therefore, despite the similar binding specificities of these ApiAP2 proteins, we predict that they carry out distinct regulatory functions in the parasite. There are several established features that can modulate binding specificity of a TF, including: DNA sequence context/shape, interactions with cofactors, and chromatin environment. In this project, we have applied both in vitro and in vivo approaches to identify how sequence preferences are established during parasite development by probing the effects of cis- and trans- regulation on TF binding. We have used a uniquely designed, high-throughput genomic context protein binding microarray (gpPBM) to examine the role of sequence context on binding specificity of each paralogous ApiAP2 TFs. Our results indicate that sequence context plays a significant role on binding site recognition. Complementary in vivo approaches such as chromatin immunoprecipitation followed by sequencing (ChIP-seq) and protein immunoprecipitation followed by mass spectrometry (IP-MS), were also applied to investigate temporal association with specific genomic loci and interactions with cofactors/protein complexes in the context of the nuclear environment. Our results illustrate that paralogous ApiAP2 proteins determine which genomic regions to bind based on a combination of factors, thereby contributing to a better understanding of the complex gene regulatory network governing P. falciparum development.

142A Interaction of Signaling Lymphocytic Activation Molecule Family 1 (SLAMF1) receptor with Trypanosoma cruzi is strain-dependent in mice modulating the immune response. Cristina Poveda1, Alfonso Herreros-Cabelló1, Francisco Callejas-Hernández1, Jesús Osuna-Pérez1, María C. Maza1, Carlos Chilón-Marinas1, Jossela Calderón1, Konstantinos Stamatakis1, Manuel Fresno1,2, Núria Gironés1,2 1) Centro de Biología Molecular Severo Ochoa (CSIC-UMA), Madrid, Spain; 2) Instituto Sanitario de Investigación Princesa, Madrid, Spain.

Chagas disease is an important problem of public health caused by the intracellular parasite Trypanosoma cruzi (T. cruzi). Clinically there are 2 main phases, the acute phase characterized by local inflammation and fever, and chronic which can be asymptomatic or with cardiac, digestive and/or both clinical symptoms. Parasite show high genetic variability and 6 different Discrete Units (DTUs) have been established. Despite experimental infections in mice with
different parasite strains produce distinct symptoms underlying different pathogenic mechanisms, results in humans do not clearly support this hypothesis. Thus, the immunopathology is still not completely understood.

We previously reported that Signaling Lymphocytic Activation Molecule 1 (SLAMF1 or CD150), controls susceptibility to T. cruzi infection with the virulent Y strain. In this work we investigated the parasitological and immunological effects of SLAMF1 deficiency in vivo with BALB/c and Slamf1-/-- mice infected by two representative parasite strains with divergent behavior, Y and VFRA strain, regarding to parasite load and their effect on macrophage NOX2 expression and ROS production.

The results showed that in the absence of SLAMF1 the immune response protected mice from the otherwise lethal Y infection favoring a pro-inflammatory response likely involving CD4, CD8, dendritic cells and classically activated macrophages. In the case of VFRA, no major changes were observed in the absence of SLAMF1. In Slamf1-/-- mice infected with Y strain, parasite load decreased in heart, intestine, spleen and liver compared to BALB/c, a fact that did not happen with VFRA strain, except for the liver, in which increased suggesting that SLAMF1 plays a very important role in this organ for the control of parasite replication.

In conclusion, our results indicate an important role of SLAMF1 interaction with T. cruzi infection that regulates the immune response. Y strain seems to depend on SLAMF1 to establish an appropriate infection, while this could not be such critical in the case of VFRA strain. However, the ultimate cause of these differences between parasite strains remains not completely understood, and the differential expression of T. cruzi surface molecules interacting with SLAMF1 may explain it. Therefore, further studies will focus in the identification of parasite molecules involved in SLAMF1 interaction to explain the immunopathogenesis of the disease.

143A The essential roles of the paralogous CAF1-CC4-NOT complex scaffold, NOT1-G, in sexual development and transmission in Plasmodium yoelii. B. Joanne Power1, Kevin J. Hart 1, Scott E. Lindner1) 1) Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, Pennsylvania, USA.

Plasmodium spp. parasites, the causative agents of human malaria, have adapted complex molecular mechanisms to control gene regulation to navigate between, and develop in, both mammals and mosquitoes during their life cycle. One such regulatory mechanism is the control of mRNA degradation via the canonical, eukaryotic CAF1-CC4-NOT complex. Previously, our lab demonstrated the critical roles of the deadenylases of this complex, CAF1 and CC4, in gametocyte maturation and transmission using the rodent malaria model, Plasmodium yoelii.

Here we show that in contrast to all other sequenced eukaryotes, Plasmodium and other members of the Acanthocephala class of apicomplexans, have evolved two NOT1 paralogues, which we term NOT1 (PyNOT1_Y_1027900) and NOT1-G (PyNOT1_Y_0945600). In P. yoelii, deletion of pynot1g resulted in a mild growth defect at e axereal blood stages, with an overcommitment to the production of gametocytes that were unable to mature. A genetic cross with wild-type P. yoelii 1XNL also revealed that, though female gametocytes were produced, both pynot1g- male and female gametocytes were infertile. Total comparative RNA-seq of wild-type and pynot1g- gametocytes revealed genome-wide changes in mRNA abundance in agreement with a failure to produce mature gametocytes.

As the only bioinformatically identifiable domain that differs between these paralogues is a predicted tristetraprolin-binding domain (TTPbd) present in PyNOT1-G, we created a variant of PyNOT1-G lacking this domain. In contrast to pynot1g- parasites, the PyNOT1-G(TTPbd-) line had no defect in sexual blood stage growth nor in the production of exflagellating male gametes but still maintained a strong transmission defect. Together, this indicates that Plasmodium has evolved this NOT1-G paralogue to be essential for both commitment to and development through gametocytogenesis, and that its TTP-binding domain is critical for efficient transmission to mosquitoes.

144A Functional characterization of two RAP proteins essential for the mitochondrial biogenesis of P. falciparum asexual stages Thomas Hollin1, Steven Abel1, Alejandro Falla2, Charisse F. Pasaje2, Jay S. Kirkwood1, Laurence Florens3, Jacquin C. Niles2, Karine G. Le Roch1 1) University of California, Riverside, USA; 2) Massachusetts Institute of Technology, Cambridge, USA; 3) Stowers Institute for Medical Research, Kansas City, USA.

In eukaryotes, RNAs interact with RNA-binding proteins (RBPs) to regulate a wide range of essential processes such as splicing, stability, localization and translational efficiency. In the malaria parasite, Plasmodium falciparum, our understanding of these mechanisms is incomplete and represents a critical brake to fight malaria. While multiple RNA-binding domains are conserved in eukaryotes, some of them seem to have specific expansions. This is particularly true for RNA-binding domain abundant in Apicomplexa or RAP proteins, enriched in Plasmodium and Toxoplasma among others. To investigate the role of these RAP proteins in the development and survival of P. falciparum, we first selected two predicted RAP proteins, RAP01 and RAP14 that were highly expressed throughout the parasite life cycle. Using CRISPR-Cas9 inducible transgenic lines, we showed that these proteins localize in the mitochondria and are both essential to the intraerythrocytic developmental cycle of P. falciparum. Transcriptomics and metabolomics assays demonstrated that the downregulation of these RAP proteins affects RNA metabolism as well as distinct plastid pathways. High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (or eCLIP-seq) confirmed the involvement of these proteins in the binding of specific mitochondrial RNA, validating their role in RNA biology inside the parasite plastid. As a whole, our study confirms that both of these RAPs are essential to the mitochondria biogenesis and represent excellent novel drug targets against malaria.

145A Delving in one carbon metabolism in the parasite Leishmania through a genomic Mut-seq screening Sophia Bigot1, 2, Philippe Leprohon2, Marc Ouellette1, 2 1) Laval University, QC, Québec, Canada; 2) Infectiology Research Center, QC, Québec, Canada.

Context

Studies of Leishmania resistant to the model antifolate drug methotrexate (MTX) has illustrated major differences in one carbon metabolism between the parasite and its host. Some of those differences could be exploited. Genomic screens are now allowing holistic views of metabolic pathways and we apply here our recently optimised Mut-seq screen with MTX selection to further our understanding of one carbon metabolism in Leishmania.

Method

Mut-seq consists in chemical mutagenesis (with ethyl methanesulfonate) of a Leishmania population and its selection on plates containing MTX. Resistant clones are characterized by next-generation sequencing and recurrent mutations (single nucleotide polymorphisms (SNPs), copy number variations), highlighted by a bio-infomatics pipeline, in independent mutants are pointing at likely candidates. Candidate mutations are further studied by molecular means.

Results

Twenty clones of L. major with a 2-400-fold decrease in MTX susceptibility in comparison to wild-type cells were sequenced. Recurrent mutations (SNPs, gene deletion) were observed in dihydrofolate reductase thymidylate synthase (DHFR-TS), pteridine reductase 1 (PTR1), several transporter of the folate bipterin transporter (FBT) family, as well as several genes involved in folate metabolism (FGPS, SHMT), as well as genes never associated with folate metabolism. We are currently validating the role of these mutations in MTX resistance using gene editing strategies.

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Conclusion
A single Mut-seq screen and the sequencing of 20 MTX resistant clones has allowed to map all known genes involved in folate metabolism and has potentially highlighted novel genes as well. Mut-seq is a powerful tool to further our understanding of one carbon metabolism in *Leishmania*.

**146A The mammalian fibrinolytic system facilitates sporozoite infection** Thiago Silva1, Andrea Radtke1, Amanda Babalab2, Tales Pascin1, Alison Roth3, Brandon Pybus3, Photini Sinnis2, Marcelo Jacobs-Lorena2, Joel Vega-Rodriguez1 1) National Institutes of Health; 2) Johns Hopkins University; 3) Walter Reed Army Institute of Research.

Plasmin is the main effector protease of the mammalian fibrinolytic system. Besides fibrin polymers, plasmin cleaves a broad spectrum of substrates including components of the extracellular matrix such as collagen, fibronectin, and laminin. Different pathogens hijack plasmin to initiate infections, as this protease facilitates cell migration across proteaceous matrices and mediates immune evasion by cleaving antibodies and complement proteins. Recently, our group found that *Plasmodium falciparum* requires plasmin to infect the mosquito midgut, in a mechanism that involves the recruitment and activation of plasminogen at the surface of sexual stages. Here we explore the hypothesis that plasmin could facilitate the infection of the mammalian host by sporozoites. We found that *P. berghei* sporozoites bind plasminogen and plasminogen activators on their surface, where plasminogen is activated into plasmin. Sporozoites expose on its surface enolase and GAPDH as receptors for plasmin. Moreover, surface-bound plasmin promotes sporozoite transmission by facilitating parasite migration across the extracellular matrices of the dermis and of the liver. Our results show a new mechanism for plasmin-mediated sporozoite migration through proteinaceous matrices. Disruption of the sporozoite interaction with plasmin is a potential molecular target to hinder *Plasmodium* infection of the mammalian host.

**147A Comprehensive *P. falciparum* RNA-protein interactome identification: beyond conventional crosslinking** Megan Gliozzi1, Heather Painter1 1) U.S. Food & Drug Administration, Center for Biologics & Evaluation Research, Division of Bacterial, Parasitic & Allergic Products, Silver Spring, MD.

The emergence of multidrug resistance greatly threatens progress in the prevention and control of human malaria infection, creating serious need for the development of novel antimarial and transmission-blocking strategies. However, identifying promising targets of intervention within the malaria parasite’s complex lifecycle has been challenging as the molecular drivers of lifecycle progression remain incompletely characterized. It has been well established that post-transcriptional regulation is essential to *Plasmodium* development and disease transmission, making RNA-binding proteins (RBPs) attractive targets of intervention. Yet, due to lack of in vivo methodologies that identify proteins directly bound to RNA and reveal their cognate motifs, characterization of RNA-protein interactions in *P. falciparum* has been limited. Here, we optimize the parameters of photocrosslinking and reported crosslinking (PAR-CL) (PMID: 22658674) for use in identifying RBPs and their target RNAs involved in the RNA-protein interactome throughout the intraerythrocytic development cycle (IDC) of *P. falciparum*. To accomplish this, we utilize a transgenic *P. falciparum* strain that has been genetically altered to salvage thiol-modified uracil (PMID: 28416533), enabling photocrosslinking (365nm) covalent crosslinking and capture of zero-distance RNPs bound to thiolated RNA. Isolation of crosslinked RNA-RBP complexes is achieved using two distinct RNA purification methods; RBPs complexed with any RNA species are captured using silica-based purification, while mRNA-associated RBPs are enriched using oligo d(T) pulldown. Following protein-RNA complex capture, mass spectrometry and novel sequencing techniques will be used to identify both protein and cognate RNA-interacting motifs. Our results will provide the first comprehensive network of RNA-protein interactions throughout the IDC and will begin to elucidate RBPs critical for lifecycle progression. Ultimately, this method provides a new and powerful tool for the study of RNA-protein interaction in *P. falciparum* and will aid in our understanding of protein-RNA dynamics throughout development and transmission.

**148A Importin α of *Trypanosoma cruzi*: A nucleolar protein which binds a classical nuclear localization signal of the bipartite type.** Israel Canela1, Ana Cevallos1, Imelda López1, Roberto Hernández1 1) UNAM Mexico.

Importin α of *Trypanosoma cruzi*: A nucleolar protein which binds a classical nuclear localization signal of the bipartite type.

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*Trypanosoma cruzi* is a member of the protist kingdom and so a unicellular eukaryotic organism. As part of the phylum Euglenozoa *T. cruzi* is classified within the family Trypanosomatidae. This microorganism is the etiological agent of Chagas disease, and also, an interesting biological model because of its atypical cellular structures. Importin α is a soluble transport factor of the classical pathway for nuclear transport of proteins in eukaryotes which has not been studied in any member of the Trypanosomatidae family. In this work we fused the ortholog gene encoding TclImportin α of *T. cruzi* to the coding sequence of EGFP fluorescence protein. Epimastigotes from transgenic cultures with this gene fusion, showed a fluorescent signal in the nucleolus, and additional dots close to the nuclear periphery of epimastigotes at the exponential growth phase. Whereas in the stationary phase, aged epimastigotes and metacyclic trypomastigotes depict a dispersed fluorescent signal in the nucleolus. On the other hand, a 6xHis tagged version of TclImportin α was found able to bind the bipartite nuclear localization signal of the cargo protein TcRPA31 (TcRPA31-EGFP), which is an essential and specific RNA polymerase I subunit in trypanosomatids. Additionally, the subcellular localization of mutant versions of this nuclear transport factor will be presented.

**149A Cryptosporidium parvum** exports proteins into the cytoplasm of the epithelial host cell Jennifer Duma1n1, Adam Sateriale1, Alexis Gibson1, Jaclyn Riley1, Amita Reddy2, Emma Hunter1, Justin Boddey3, Brad Sleebs3, Boris Striepen1 1) University of Pennsylvania, Philadelphia, PA; 2) University of Georgia, Athens, GA; 3) Walter and Eliza Hall Institute, Parkville, VIC Australia.

The parasite *Cryptosporidium* is responsible for diarrheal disease in young children causing death, malnutrition, and stunted growth. *Cryptosporidium* invades enterocytes where it develops in a specialized intracellular but extraplastidicniche. Infected cells exhibit profound changes in cellular morphology, physiology and transcriptional activity in response to the parasite. Host-targeted parasite effectors have been hypothesized to be driving agents of these changes; however, no such proteins have been identified. Using CRISPR/Cas9 driven homologous recombination, we epitope tagged the endogenous loci of the *T. cruzi* tagpartite nuclear importin α of *T. cruzi* was found able to bind the bipartite nuclear localization signal of the cargo protein TcRPA31 (TcRPA31-EGFP), which is an essential and specific RNA polymerase I subunit in trypanosomatids. Additionally, the subcellular localization of mutant versions of this nuclear transport factor will be presented.

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The most common species of trypanosomes that are responsible for animal African trypanosomiasis (AAT) in sub-Saharan Africa including Ghana are *Trypanosoma brucei brucei*, *T. vivax* and *T. congolense*. Despite the major impact of the disease on livestock production which affects food and economic security there is no study on lifetime trypanosome infections in cattle. Here, the trypanosome species found in cattle throughout their natural infection cycle were characterized over a two year period. Two herds of cattle (20 each) at Accra and Adidome were selected on the basis of geographical location, tsetse fly density, trypanosomiasis prevalence and the breed and age of cattle at the sites. The infecting trypanosomes were identified in blood collected at approximately four to five-week intervals and characterized using multiplex nested polymerase chain reaction (PCR) targeting a portion of the trypanosome tubulin gene cluster and next generation sequencing. Our data shows that cattle were infected throughout the natural infection cycle at both study sites with cases of mixed infections. *T. vivax*, *T. brucei*, *T. theileri* and *T. congolense* were the major infecting species at both study sites with *T. vivax* being predominant [incidence rate of 84% (282 samples) for Adidome and incidence rate of 70.3% (335 samples) for Accra]. *T. vivax* was also the principal trypanosome species existing within both young and old aged cattle, among males and females and the different cattle breeds at both study sites. Interestingly, apart from infections with trypanosomes various species of tick-borne parasites such as *Babesia bovis*, *Theileria wilfii* and *Theileria mutans* were also detected. Data from this study has given an insight on natural trypanosome infection in the wild and the burden of coinfection with other blood pathogens. The data generated will inform policy makers of better control measures for the affected areas.

5′ to 3′ decay is the major mRNA degradation pathway in many organisms, including trypanosomes. First, the m7G cap is removed by the nudix domain hydrolase Dcp2, which is part of a larger decapping complex. Trypanosomes lack homologues to all decapping complex proteins and we have recently identified an Apah-like phosphatase (TbALPH1) as the major mRNA decapping enzyme of trypanosomes. In vitro, TbALPH1 has mRNA decapping activity in a wide range of conditions without cap-type preference and surprisingly, largely independent on its C and N terminal domains that embed the catalytic domain. In vivo, these C- and N-terminal extensions determine enzyme localisation and protein interactions, likely regulating enzyme specificity. Even though Apah-like phosphatases are present in all eukaryotic super-groups, our phylogenetic studies strongly suggest that their usage in mRNA decapping is unique to kinetoplastida of Toxoplasma. Second, we used bio-orthogonal chemistry in semi-permeabilized parasites to label, capture and identify direct CDPK1 substrates. First, we monitored CDPK1 signaling in live cells at sub-minute resolution to determine factors exhibiting CDPK1-dependent phosphorylation. Second, we used bio-orthogonal chemistry in semi-permeabilized parasites to label, capture and identify direct CDPK1 substrates. Our results provide a dynamic analysis of calcium-regulated secretion and further our understanding of CDPK1’s role by identifying its direct and indirect targets. Global time-resolved monitoring of Toxoplasma’s phospho-proteome during secretion has identified novel candidates that may control secretion. In addition, these results reveal regulation of known effectors of secretion, which include factors involved cyclic-nucleotide, lipid, and calcium signaling. Ongoing work is focused on the functional characterization of novel CDPK1 targets associated with vesicular trafficking and membrane fusion.

The human malaria parasite *Plasmodium falciparum* relies on lipids to survive, making its lipid metabolism an attractive drug target. The lipid phosphatidylyserine (PS) is usually confined to the inner leaflet of the red blood cell membrane (RBC) bilayer; however, some studies suggest that infection with the intracellular parasite results in the presence of this lipid in the RBC membrane outer leaflet. PS in the outer leaflet acts as a recognition signal to phagocytes in other systems, but it has not been experimentally determined whether PS contributes to phagocytic recognition of *P. falciparum*-infected RBCs. Here, we investigated the enzymatic reactions responsible for maintaining asymmetry between membrane leaflets, and found that in parasitised RBCs the maintenance of membrane asymmetry was partly dissolved, and PS was increased in the outer leaflet. We examined the underlying causes for the differences between uninfected and infected RBCs, and found that calcium levels increased in the RBC cytoplasm, whereas membrane cholesterol was efficiently depleted from the erythrocyte plasma membrane. We explored the resulting effect of PS exposure on enhanced phagocytosis by monocytes, and provide evidence that infected RBCs must expend energy to limit phagocyte recognition. Together, these findings underscore the pivotal role for PS exposure on the surface of *Plasmodium falciparum*-infected erythrocytes in *in vivo* interactions with the host immune system, and provide a rationale for targeted antimarial drug design.
Trypanosoma brucei causes human sleeping sickness and frequently alters its major surface antigen (VSG) that is exclusively expressed from one of 13 expression sites (ESs) to counter the host immune response. The long non-coding RNA, TERRA, was discovered as a heterogenous species transcribed from telomerases that were traditionally considered 'silent'. TERRA has been shown to be an important player in telomere protection, regulation of telomere length, and telomere recombination in mammalian and yeast cells and in gene expression regulation in mouse ES cells. TERRA expression level is cell cycle regulated in human and yeast. In T. brucei, TERRA is expressed from the telomere downstream of the active VSG ES as a result of RNA polymerase I readthrough transcription, unlike that in other TERRA-expressing organisms where it is transcribed by RNA polymerase II. Our lab previously showed that the telomere complex plays key roles in antigenic variation, and fine-tuning TERRA levels is important for T. brucei fitness. However, TERRA functions are still not fully understood. In this study, we investigated the subcellular localization of TERRA using RNA FISH. We observe that TERRA can form one or more foci, but G1 cells frequently have only one TERRA focus. We demonstrate a unique cell cycle-dependent TERRA metabolism in T. brucei, unlike that in human and yeast cells. In addition, TbTERRA directly binds to TERRA and suppresses the TERRA level. Strikingly, The TERRA-binding interface of TbTERRA resides in its Myb domain but is independent of its duplex TTAGGG repeat binding activity, which is unique among TRF homologues. TbTERRA-depletion not only affects the TERRA foci pattern but also leads to enhanced yH2A level and more telomeric R-loops, suggesting that the increased amounts of TERRA and telomeric R-loops contribute to more telomeric DNA damages.

1558 Diversity in the intrinsic apoptosis pathway of nematodes Neil Young1, Tiffany Harris3, Marco Evangelista2, Sharon Tran2,3, Merridee Wouters2,4, Tatiana Soares da Costa3, Nadia Kershaw5, Robin Gasser1, Brian Smith3, Erinnia Lee2,3, W. Douglas Fairlie2,3 1) University of Melbourne; 2) Olivia Newton-John Cancer Research Institute; 3) La Trobe University; 4) University of Sydney; 5) Walter and Eliza Hall Institute of Medical Research.

Studies of the free-living nematode C. elegans informed how the BCL-2-regulated apoptosis pathway of humans is regulated. However, subsequent studies showed C. elegans apoptosis has several unique features compared with that of humans. There have been no detailed analyses of apoptosis regulators in other nematode species to date. Here, we discovered C. elegans-orthologues in 89 free-living and parasitic nematode taxa representing four distinct evolutionary clades (I, II, III, and V). Unlike in C. elegans, 15 species possess multiple (two to five) BCL-2-like proteins, and some species did not have any recognisable BCL-2 sequences. Functional studies provided no evidence that BAX/BAK proteins have evolved in nematodes. Structural studies on a non-canonical BCL-2 protein from the basid clade I revealed it lacks a functionally important feature of the C. elegans orthologue. Clade 1 CED-4/AAFAP-1 proteins also possess WD40-repeat sequences associated with apoptosis assembly, not present in C. elegans, or other nematode taxa studied.

1568 Identifying a specific inhibitor of the invasion of red blood cells by Plasmodium falciparum Madeline Dans1,2, Greta Weiss1, William Nguyen3, Zahra Razook1,2, Sonya Mehra1,2, Maria Gancheva4, Ornella Romeo4, Danny Wilson4, Chris Tonkin3, Brad Sleebs3, Brendan Crabb1,5, Alyssa Barry1,2, Tania de Koning-Ward2, Paul Gilson1 1) Burnet Institute, Melbourne, VIC, Australia; 2) Deakin University, Geelong, VIC, Australia; 3) Walter and Eliza Hall Institute, Parkville, VIC, Australia; 4) The University of Melbourne, Parkville, VIC, Australia; 5) The University of Melbourne, Parkville, VIC, Australia.

With emerging resistance to frontline treatments, it is vital that new drugs are identified to target the malaria parasite, Plasmodium falciparum. A critical process during the parasites’ asexual lifecycle is the invasion and subsequent egress of red blood cells (RBCs); an action that requires many unique parasite ligands, receptors and enzymes that could be exploited as druggable targets. To identify potential inhibitors, we screened the Medicines for Malaria Venture (MMV) Pathogen Box, a 400 compound library comprised of drugs against neglected tropical diseases, including 125 with antimalarial activity. In the presence of the compounds, we utilised transgenic parasites expressing the bioluminescent reporter, Nanoluciferase, to measure inhibition of parasite egress and invasion. At a concentration of 2 μM, we identified 11 compounds that reduced egress to <60% and 24 compounds that reduced invasion to <10%. We have further characterised 11 of these inhibitors through cell-based growth assays and live cell microscopy, and have found them to either inhibit egress, directly inhibit parasite invasion or cause general growth defects that manifests as invasion inhibitory effects. We have found a sulfonylpiperazine, termed MMV020291, to be the most invasion specific inhibitor, blocking successful merozoite internalisation within human RBCs and having no substantial effect on the rest of the cell cycle. To identify the potential target(s) of MMV020291, we performed resistance selection by exposing parasites to high concentrations of the compound. Whole genome sequencing and bioinformatic analysis was conducted on three MMV020291 resistant populations revealing three non-synonymous single nucleotide polymorphisms (SNPs) in two genes involved in generating the force required for parasite invasion of RBCs. Currently, we are aiming to confirm these targets by genetically engineering the SNPs into wildtype parasites using CRISPR-Cas9. In conjunction to the target biology, we are also collaborating with medicinal chemists that have created analogues of MMV020291, achieving activity in the low nanomolar range. This study should provide insight into the mechanisms underlying parasite invasion of RBCs, in addition to advancing the development of a novel antimalarial.

1578 Imiquimod targets toxoplasmosis through modulating host Toll Like receptor-MbD8 signaling Maguy Hame1, Carine Deleuze-masquefa2, Pierre Antoine Bonnet2, Jean-François Dubremetz3, Marwan El-Sabban4, Hiba El-Hajji1 1) department if experimental pathology, American University of Beirut ; 2) University of Montpellier IBMM; 3) UMR 5235 CNRS; 4) Department of Anatomy, American University of Beirut.

Imiquimod binds to TERRA and suppresses the TERRA level. Strikingly, The TERRA-binding interface of TbTERRA resides in its Myb domain but is independent of its duplex TTAGGG repeat binding activity, which is unique among TRF homologues. TbTERRA-depletion not only affects the TERRA foci pattern but also leads to enhanced yH2A level and more telomeric R-loops, suggesting that the increased amounts of TERRA and telomeric R-loops contribute to more telomeric DNA damages.

158B 18S rDNA Sequence-Structure Phylogeny of the Trypanosomatida (Kinetoplastea, Euglenozoa) with Special Reference on Trypanosoma by Markus Engstler1, Matthias Wolf2 1) Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg; 2) Lehrstuhl für Bioinformatik, Biozentrum, Universität Würzburg.

Trypanosomes cause African sleeping sickness and Chagas disease in South America, and Leishmanio species infect and harm hundreds of thousands of people each year. Without any doubt, it is important to study the evolution of this group of organisms. Using SSU 18S ribosomal RNA gene sequence-structure data, we reconstructed the phylogeny of a comprehensive sampling of trypanosomes evaluated in the context of trypanosomatid diversity. The available secondary structures of the 18S rRNA from Trypanosoma cruzi and Trypanosoma brucei were used as templates to predict the secondary structures of other
Unexpected rate of Alternative Splicing Events in the Early Strobilar Development of *Echinococcus granulosus* Mohammad Ali mohamad1, Mehdi Mansouri2, Majid Fashi Harandi1 1) Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran; 2) Department of Agricultural Biotechnology, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

*Echinococcus granulosus*, is a widespread parasitic zoonosis causing major medical and economic burden in endemic regions. The ability of *E. granulosus* protoscoleces to respond quickly to environmental changes in the host body requires adaptations in different levels of gene expression regulation. Alternative splicing (AS) and the related cellular functions are one of the major fields of the post-genomic. High frequency and potential versatility of AS in many fundamental biological processes have been poorly studied in tapeworms, therefore we investigated AS events and its potential biological effects in *E. granulosus*.

Whole transcriptome sequencing data used in this study were generated by an earlier study (SRP131874). Four groups of samples were prepared for RNA-seq library construction, i.e. none treated (NT), pepin treated (PEP), and pepin treated after 12 (12h) and 24 hours (24h). The high-quality trimmed reads were mapped to the published genome of *E. granulosus* (PRJEB1211) by using STAR aligne. Different types of AS events were identified using rMATs. Functional annotations and gene ontology of differential AS (DAS) genes were performed by using Blast2GO software. AS events were experimentally validated by performing PCR on the protoscoleces cDNAs with a specific primer for each gene.

We found AS events in 38.1% of the genes in the protoscoleces. In this study, 3,904 unique Gene ID from 10,245 *E. granulosus* coding genes was predicted with at least one AS event. In this regard, 68 unique gene was significant between all groups of early strobilar development of *E. granulosus*. KEgg pathway analysis revealed a number of genes belonging to the different components of spliceosome complex undergoing AS. These genes were tended to common SR proteins, U1 related factors, U2 related factors, Complex A specific factors and other splicing related proteins. The number of genes undergoing AS is in accordance with the frequency of AS events in other platyhelminths. Although total number of exon/introns in *E. granulosus* is much different from other eukaryotes, the number of genes undergoing AS in *E. granulosus* still seems unusually high. This number of AS events in the regulatory mechanisms of gene expression confirms the essential rapid adaptation process for the parasite at the molecular level in different environments. More in-depth studies are required to explain this high level of AS genes in *E. granulosus* metacestodes.

Altered functionalities of two organellar DNA base excision repair enzymes of *Plasmodium falciparum* Anupama Tiwari1, Jitendra Kuldeep1, Mohammad Iman Siddiqi1, Saman Habib1 1) Council of Scientific and Industrial Research-Central Drug Research Institute.

The maintenance of integrity of organellar genomes of the mitochondria and apicoplast in *P. falciparum* is essential for overcoming harmful effects of several endogenous and exogenous DNA damaging agents as well as for repairing errors generated during replication. Mutation accumulation in organellar DNA is likely to impact critical proteins encoded by these genomes. Further, altered DNA repair pathways have also been linked with the mutator phenotype of drug resistant *Plasmodium* strains. We explored the parasite genome for putative base excision repair enzymes with an organellar targeting element, and present localization and biochemical characterization of two BER enzymes: a homolog of bacterial ENDO IV, PfApn1 and a homolog of *Arabidopsis thaliana* 5′-3′ exonuclease, PfExo5′-3′.

Putative BER pathway enzymes, PfApn1 and PfExo5′-3′ were expressed as recombinant proteins in *Escherichia coli*. Immuno-localization using specific antibodies generated against the proteins indicated mitochondrial targeting of PfApn1 whereas PfExo5′-3′ localized to the apicoplast. PfApn1 is a divalent metal ion-dependent AP endonuclease which has three conserved metal binding sites. AP-site cleavage assay and CD spectra confirmed the requirement of both Zn2+ and Mn2+ ions for optimum activity and active conformation of PfApn1, respectively. Metal determination of PfApn1 revealed two Zn2+ and one Mn2+ bound per molecule enzyme; mutation of the third metal binding site residue H542N rendered the enzyme inactive while abolishing Mn2+ binding thus suggesting a critical role for Mn2+. Molecular dynamics simulation further supported the fact that Mn2+ occupies the third metal binding site but, unlike known homologs in other organisms, it completely lacks 3′-5′ exonuclease activity and nucleotide incision repair function. Functional characterization of PfExo5′-3′ revealed that it hydrolyzed nucleotides from 5′ end of dsDNA (5′ recessed, gapped, blunt-end) indicating a wide range of substrates. PfExo5′-3′ also hydrolysed ssDNA templates in both 5′-3′ and 3′-5′ direction, suggestive of its bidirectional exonuclease activity on ssDNA templates. Its additional 5′ flapping activity suggests its role in long-patch BER in the apicoplast. Our study provides the first evidence of functional BER pathway in parasite organelles.

The lateral diffusion of non-VSG, GPI-anchored proteins of *Trypanosoma brucei* Thomas Mueller1, Nicola G. Jones1, Elisabeth Meiser1, Olivia J. Macleod2, Mark Carrington2, Susanne Fenz1, Markus Engstler1 1) Biozentrum, Universität Würzburg, Germany; 2) Department of Biochemistry, University of Cambridge, UK.

Unravelling the mechanisms of immune evasion of the unicellular parasite *Trypanosoma brucei* is a topic of ongoing research. Their dominant evasion strategy is antigenic variation, mediated by the presence of a dense coat of variant surface glycoprotein (VSG), and frequent changes of this coat. A less common contributor to immunity is the factor H receptor (FHR), a low abundance, glycosylphosphatidylinositol (GPI)-anchored protein in the outer membrane of the blood- and insect-stage of the parasite. This receptor binds factor H (FH) from the host to prevent opsonisation by the complement system. To gain insight into the diffusion properties of the FHR, we performed in a controlled environment, consisting of supported lipid bilayers and the fluorescently labelled receptor. Addition of the ligand FH to the FHR did not influence the diffusion properties of the receptor. In all experiments, the trajectories of the diffusing proteins revealed slow and fast diffusing populations as well as frequent changes between these, which were independent of membrane properties. This might be related to protein-lipid interactions on the sparsely populated membranes. We now plan to study the FHR, and other low abundance surface proteins, in mixed coats with defined amounts of VSG, to characterise the complex and highly mobile surface coat of *T. brucei*. Our goal is to understand the physical processes underlying the immune evasion of trypanosomes.
Leishmaniasis is a disease caused by the protozoan parasite of the genus *Leishmania* and its endemic in Africa, Asia, southern Europe and the Americas. It is transmitted by sandfly vectors and it is associated with poverty. Its coinfection with HIV has increased its fatality wherever it is found and with the development of a vaccine against it still being a mirage, the most reliable intervention point against this disease remains chemotherapeutics. However, the current anti-leishmanial drugs are reported to be highly toxic, requires long term administration regimen, not readily accessible and costly. Here, we showed the anti-leishmanial activity in *vitro* and the likely mode of action of some 68 Medicine for Malaria Venture (MMV) compounds against the promastigotes and amastigotes stages of the *Leishmania donovani* parasites. The growth inhibitory concentrations (IC50) obtained ranged between 10 nM and 95 µM. Twenty-four (24) of the 68 compounds were tested for their cytotoxicity against RAW macrophage Cell lines and a selectivity index range of 0.03 to 455 was observed. The growth kinetic and growth reversibility profiles of twenty (20) of the compounds were indicative of a cytostatic effect, while another four (4) showed a cytoidal effect on the parasites, some mediated programmed cell death through apoptosis and altered the cell cycle progression of the parasites by causing growth arrest at either the G0-G1, S and G2-M phases of the parasite cell mitotic division. Morphological analysis using fluorescence microscopy revealed obvious distortion in the mitochondrion integrity (60%) and the absence of DAPI-stained kinetoplast DNA (30%). Our findings present useful therapeutic potentials of these compounds in Leishmaniasis.

**163B Divergent acyl carrier protein mediates mitochondrial iron-sulfur cluster metabolism in malaria parasites** Seyi Falekun1, Yasi Jami2, Hahnbeom Park3, James Wohischlegel2, Paul Sigala1 1) Department of Biochemistry, University of Utah; 2) Department of Biological Chemistry, University of California Los Angeles; 3) Institute for Protein Design, University of Washington.

*Plasmodium falciparum* malaria parasites are early-diverging eukaryotes with many unusual metabolic adaptations. In contrast to humans, the *Plasmodium* mitochondrion lacks type II fatty acid biosynthesis (FASII) enzymes yet curiously retains a divergent acyl carrier protein (mACP) missing the 4-phosphopantetheine (4-Ppant) group required for acyl transport. We report that ligand-dependent knockdown of mACP expression is lethal to parasites, indicating an essential, FASII-independent function. This lethal phenotype is rescued short-term by decyl-ubiquinone, suggesting a dominant dysfunction of the mitochondrial electron transport chain (ETC). Biochemical studies reveal that mACP binds and stabilizes the Isd11-Nfs1 complex that produces Fe-S clusters, and mACP knockdown causes loss of the Fe-S cluster-dependent Rieske protein in ETC Complex III. This work highlights the ancient, fundamental role of ACP in mitochondrial Fe-S cluster biogenesis, provides a new molecular function for mACP function independent of FASII, and identifies *aPlasmodium*-specific adaptation that can serve as a new antimalarial drug target.

**164B Role of mitochondrial thioredoxins in promoting survival of Toxoplasma gondii under redox stress** Mariana Silva1, Lewis King2, Richard Hartley2, Lilach Sheiner1 1) Federal University of Uberlandia; 2) University of Glasgow.

Toxoplasma gondii is an intracellular parasite that belongs to Apicomplexa phylum. To survive and proliferate in a variety of host environment, *T. gondii* uses different mechanisms to maintain infection. Maintaining appropriate cellular functions in the face of redox imbalance is one of the critical challenges for *T. gondii*, and yet these mechanisms remain largely unknown. Some organisms have enzymes, named thioredoxins (Trxs), that controls the activity and localisation of proteins in respond to redox changes in cellular compartments through disulfide exchange. *T. gondii* possesses an unusually high number of thioredoxins, most predicted essential for its survival per the genome wide CRISPR screen, yet their role is not known. I will present novel Trxs I identified in *T. gondii* tachyzoites using bioinformatics, and the progress made towards understanding their function. Among 55 thioredoxin candidates are expected to be apicoplast or mitochondrial. Using endogenous tagging and immunofluorescence I validated mitochondrial localization for 2 Trxs and work for validating others is ongoing. Finally, RNAseq analysis of *T. gondii* treated with a mitochondrial redox cyclo catalyst highlighted 3 Trxs that are likely involved in the adaptation to redox stress in this organellae. Work is ongoing to confirm this using genetic manipulation and to identify their substrate. The high sensitivity of *T. gondii* to redox imbalance present redox regulation pathways as attractive drug targets. My work provides knowledge that could contribute to the design of such strategies in the future.

**165B Development and application of loop-mediated isothermal amplification for the diagnosis of schistosomiasis in Tomefa, an endemic community in Ghana.** Isaac Owusu-Frimpong1,3, Linda B. Debrah2,3, Samuel Armo1, Edward J. Tettev1, Yvonne A. Ashong4, Naa A. Kuma1, Frank T. Aboagye1, Mike Y. Osei-Atweneboana1 1) CSIR - Water Research Institute, Accra, Ghana; 2) Kumasi Centre for Collaborative Research into Tropical Medicine (KCCR), Kumasi, Ghana.; 3) Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.; 4) Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana.

**Background**

Schistosomiasis affect all 9 regions of Ghana, putting an estimated 26.3 million Ghanaians at risk. Currently, the “gold standard” for detection of the infection in Ghana is by microscopy. However, this technique usually underestimates the levels of infections, and has resulted in the development of other improved diagnostic techniques. Loop-mediated isothermal amplification (LAMP) evolved in 2000, and compared to PCR-based assays, it is a rapid, cost-effective and ready-to-use approach. This study was performed to detect *Schistosoma mansoni* and *Schistosoma haematobium* infections using LAMP primers designed from the COX 1 gene and an end-point colour change detection system.

**Methodology/Principal findings**

This was a cross-sectional study which involved 200 stool and 150 urine samples collected from archives of an ongoing work at Tomefa, in the Ga South Municipal District of Ghana. Schistosoma-DNA was extracted from samples using a modified Zymo-Quick DNA Plus kit protocol. Consensus sequences from the COX 1 gene of the mitochondrial genome of each *S. mansoni* and *S. haematobium* were used to design LAMP primers, after alignment of sequences from the NCBI GenBank. The newly designed *Schistosoma* species-specific primers, in addition to the in-house prepared colorimetric buffer were then used in the application of the LAMP assay, after which the end-point colour change was read. The positive cases of uringenal and intestinal schistosomiasis detected by microscopy increased from 48.76% and 75% to 52% and 97%, respectively, after the application of LAMP. The lowest detection limit was estimated as 306 fg of DNA.

**Conclusion/Significance**

Good progress has been made in the development of LAMP assay for the detection of *S. mansoni* and *S. haematobium* infections in faecal and urine samples, which is potentially applicable in epidemiological surveys and resource poor-settings. This tool will aid in the accurate evaluation and monitoring of mass drug administration programs, and disease surveillance.

**166B Essential exon floxing shows that Cystein repeat modular proteins are critical for Invasion in Toxoplasma gondii** Mirko Singer1, Markus Meissner1 1) LMU Munich, Germany.
Cysteine repeat modular proteins (CRMPs) are a family of apicomplexan specific proteins. They contain a conserved core of 8 to 10 transmembrane domains and a long extracellular domain with cysteine rich adhesion domains. Plasmodium species contain four CRMPs which have been linked to oocyst egress, salivary gland invasion and liver cell invasion as well as asexual replication in red blood cells.

In Toxoplasma gondii, only two functional CRMPs are present in the genome. The Kringel domain containing protein (Kringel) linked to CRMP1 and 2 and the GCC2GCC3 domain containing protein (GCC2) with a 4000 amino acid n-terminal insertion is linked to CRMP3 and 4. Both proteins mainly localize in micronemes.

Gene deletion using DiCre with the whole GCC2 gene floxed by LoxP sites showed almost no gene excision of the 25 kbp gene. Therefore, we directly inserted a LoxP site into the last intron using Cas9. This allowed excision of the conserved core of GCC2, removing the conserved EGF-like domains and all transmembrane domains.

While some protein was still present 48 hours after DiCre activation, after 72 hours invasion was severely reduced for both Kringel or GCC2. After gene excision, parasites failed to secrete rosettes, while intracellular growth, egress and gliding motility was not affected. Generation of double tagged but single floxed parasites showed that while GCC2 localization is independent of Kringel presence, excision of GCC2 results in reduced levels of Kringel-YFP.

prior to immunization, suggesting a DNA-dependent mechanism of immune protection. We present the dose and time dependence of injected hemozoin on liver malaria inhibition was found after hemozoin immunization in BALB/c mice. However, the protective effect was ablated when hemozoin was treated with DNase 1.

168B Vps32 is required for endocytic trafficking to the lysosome in Trypanosoma brucei Nadia Barrera1, Alejandro Schojelt1,2, Guillermo Alonso1,2 1) Instituto de Investigaciones en Ingeniería Genética y Biología Molecular “Dr. Héctor N. Torres” (INGEBI- CONICET), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina; 2) Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular, Buenos Aires, Argentina.

Trypanosoma brucei orients alternates between the mammalian bloodstream form (BSF) to multiple stages in the insect vector, including the midgut procyclic form (PCF) requiring processes for adapting and restoring homeostasis during life cycle progression and infection. These processes are guided by membrane trafficking. The multivesicular bodies (MVB) are specialized late endosomes (LE) that function in targeting ubiquitinylated cell surface proteins to the lysosome for degradation and are mainly composed of proteins members of The Endosomal Sorting Complex Required for Transport (ESCRT). ESCRT is composed of four subcomplexes (0-III) being ESCRTII the most conserved among eukaryotic taxa. Vps32, the most abundant protein of ESCRT III, is an important role in cytokinesis and vesicular trafficking in Saccharomyces cerevisiae and Homo sapiens. African trypanosomes lack an morphologically well-defined MVB, but contain orthologues of the ESCRT, machinery that drives a diverse collection of membrane remodeling events. In fact, in Trypanosoma brucei TbVps23 (ESCRTI) and TbVps4 (the terminal ESCRT ATPase) are both localized to the late endosome and play a role in lysosomal trafficking. Here, we have identified the Vps32 orthologue in Trypanosoma brucei, named TbVps32, associated with endocytic compartments. By the TbVps32 silencing and inducible expression of HA-TbVps32, we addressed the TbVps32 role in vesicular transport to lysosome and cell cycle progression. Knockdown of TbVps32 by interference RNA and HA-TbVps32 inducible over-expression resulted in inhibition of cell growth. Moreover, trafficking of dextran, transferrin and DQ-BSA in endocytic pathway was impaired. Overall, we propose that TbVps32 participates in endocytic trafficking to the lysosome and is essential for Trypanosoma brucei survival.

168B Plasmodium berghei hemozoin bound to DNA confers partial protection against liver stage infection in BALB/c mice Adriano Franco1,2, David Sullivan1,2 1) Johns Hopkins Bloomberg School of Medicine, Baltimore, MD; 2) Johns Hopkins Malaria Research Institute, Baltimore, MD.

After erythrocyte invasion, Plasmodium relies on host hemoglobin as its main source of amino acids. A toxic form of heme is released during hemoglobin digestion, which is avoided by the parasite when it’s crystalized into a compound known as hemozoin. Hemozoin is released into circulation after erythrocyte lysis caused by merozoite replication. Monocytes have been known to phagocytize hemozoin during Plasmodium infection in both in vitro and in vivo assays. In vitro immune responses to hemozoin have been associated with a TLR-9 response triggered by parasitic DNA bound to the crystal. This in vitro immune effect is dependent on DNA being attached to hemozoin. Here we investigated the protective role of parasitic hemozoin during Plasmodium liver stage infection and the immune mechanism of protection. Livers and spleens were harvested from infected mice for hemozoin extraction. BALB/c mice received intravenous immunizations of 750 nmol of hemozoin. After resting for one week, the same mice were inoculated intravenously with 2000 sporozoites incorporated with a luciferase reporter gene. 42 hours post infection, parasitic liver burden was measured in photons/second using In Vivo Imaging System. An 80% liver-stage malaria infection was found after hemozoin immunization in BALB/c mice. However, the protective effect was ablated when hemozoin was treated with DNase prior to immunization, suggesting a DNA-dependent mechanism of immune protection. We present the dose and time dependence of injected hemozoin on liver stage parasite levels.

169B The role(s) of conserved motifs within the 3′ untranslated region of the variant surface glycoprotein of African trypanosomes Majed Bakari Soale1, Henriette Zimmermann1, Christopher Batram1, Nicola G. Jones1, Falk Butter2, Markus Engstler1 1) Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Würzburg, Germany; 2) Quantitative Proteomics, Institute of Molecular Biology (IMB), Mainz, Germany.

Trypanosoma brucei utilizes a dense variant surface glycoprotein (VSG) to protect itself from host immune factors. The parasite undergoes an elaborate system of antigenic variation where it expresses only a single variant of the VSG out of a repertoire of ~2000 genes. The molecular processes and the order of events governing the expression and regulation of the VSG are not very well understood.

The regulation of gene expression in trypanosomes is largely post-transcriptional. Cis-elements within the 3′ untranslated regions (3′UTR) of most eukaryotic genes are involved in post-transcriptionally regulating genes and gene products. In T. brucei, a highly conserved 16mer motif within the 3′ UTR of VSGs has been shown to modulate stability of VSG transcripts and hence its expression. This 16mer motif is however 100% conserved in all T. brucei VSGs. This unusually high conservation of a ‘stability motif’ led us to hypothesize that the motif is involved in other essential roles/processes besides stability of the VSG transcripts.

We have demonstrated that an intact 16mer is not required for the expression of wildtype VSG levels. We have further shown that the intact motif is not required for silencing of the VSG during switching and also during differentiation from bloodstream forms to procyclic forms. Crossover between the VSG and procyclin during differentiation to the insect vector stage is also unaffected in 16mer mutant parasites. This study further seeks to identify and characterize interacting partners of the motif to enable us to discern the functional significance driving the surprising 100% conservation of an RNA-motif.

170B Characterization of calcium signaling in bradyzoites of Toxoplasma gondii Yong Fu1, Kevin Brown1, Nathaniel Jones1, David Sibley1 1) Washington University School of Medicine.

Toxoplasma gondii is an obligate intracellular parasite that has evolved different developmental stages for disseminating during acute infection vs. establishing long lived chronic infection. Calcium signaling tightly regulates the lytic cycle of tachyzoites by controlling microneme secretion and motility to drive egress and invasion, while these pathways are shut off during intracellular replication. However, little is known about the role of calcium signaling pathway in bradyzoites, which are found within semi-dormant cysts. To investigate calcium signaling in bradyzoites, we generated type II ME49 strain dual-reporter parasites that express mCherry driven by the BAG1 promoter and the calcium indicator GCAmp6F. We utilized a calcium ionophore A23187 and zaprinast, a phosphodiesterase inhibitor that elevates cGMP and thus increases calcium levels, to examine calcium responses in bradyzoites within cysts from three different sources. In vitro cysts produced by alkaline induced stress in HFF cells, in vitro cysts that form naturally in C2C12 muscle cells, and ex vivo cysts harvested from...
the brains of chronically infected mice. In all cases, we observed slower kinetics and lower amplitude of calcium responses in bradyzoites compared with tachyzoites. Additionally, zaprofin induced weaker calcium responses and decreased motility of bradyzoites compared with A23187, suggesting that PKG activation may differ in bradyzoites. Stimulation by these agonists activated motility and led to escape by a minority of bradyzoites but did not lead to rupture of cyst, suggesting that the wall presents a barrier to escape. To test this idea, we utilized trypsin to disrupt cyst wall and liberate bradyzoites. Interestingly, under these conditions, released bradyzoites display calcium-dependent motility similar to tachyzoites. Overall, our findings indicate that intracellular bradyzoites exhibit reduced calcium responses, which may arise from differential permeability or differences in signaling pathways that govern release.

1718 The role of Acetyl-CoA Synthetase in \emph{P. falciparum} epigenetic control

Isadora O. Prata1, Eliana F.G. Cubillos1, Wesley L. Fotoran1, Gerhard Wunderlich1 1) University of São Paulo (USP).

The protozoan genus \emph{Plasmodium} is the causative agent of malaria, which affects the population living in developing countries in the tropics. \emph{P. falciparum} is responsible for the severest form of malaria in humans. The clinical symptoms of malaria appear during the intraerythrocytic cycle in the human host, and parasite-mediated sequestration of erythrocytes is directly related to the pathogenesis of severe malaria. The variant PIEMP1 protein family, encoded by the \emph{var} gene family, is involved in erythrocyte sequestration, which has several consequences related to severe malaria. Many processes are involved in \emph{var} gene expression control, which is mainly regulated by epigenetic factors. Here we aim to characterize the conserved enzyme Acetyl-CoA Synthetase (ACS) in \emph{P. falciparum} and also understand its role in \emph{var} gene expression control. We generated mutant lines to modulate PFACS protein by means of the DD24 stabilizing domain and its transcript through the ribosome glmS. Immunoblot exhibited that PFACS protein levels were affected by the inducible knockdown. After 24 hours of PFACS knockdown parasites started to appear unhealthy and asynchronous. In the subsequent 48 hours, PFACS absence led to parasite death, indicating that PFACS is an essential protein for blood stage \emph{P. falciparum} survival. Further, we tested a specific ACS inhibitor (iACS) in parasite cultures and observed that parasites were dying in an inhibitor dose dependent manner, presenting the same phenotype observed in our mutant lines after PFACS knockdown. Through immunoblot we noticed a huge decrease in parasite histone acetyl marks. Considering the loss of epigenetic marks, we performed a cytoadherence assay were iACS treated parasites apparently lose its \emph{var} genes profile and consequently its cytoadherent phenotype. We also tested the effects of iACS in the murine model \emph{P. berghei}, were we observed loss of epigenetic marks and a significant decrease in parasite infectivity. Hence, our results indicate that PFACS is an essential enzyme involved in epigenetic and metabolic processes, being a good target for drug development.

1728 A lysine stretch in coronin modulates directionality of gliding motility in malaria parasites

Julia Sattler1, Marie Neuhoff1, Katharina Quadt1, Friedrich Frischknecht1 1) University Hospital Heidelberg.

The rapid gliding motility of malaria parasites proceeds in contrast to slow amoeboid crawling without a change of the cellular shape. Gliding motility is essential for various steps along the complex \emph{Plasmodium} life cycle including transmission from mosquitoes to humans. To achieve their high speed, these parasites evolved dynamic and short actin filaments, that are regulated by a minimal set of actin-binding proteins, which include the actin filament-binding protein coronin. Plasmodium coronin regulates rapid motility of sporozoites, the crescent shaped parasite forms transmitted by the mosquito (Bane et al.). Here we show that a lysine- stretch within the unique region of the C-terminal part of coronin is important for proper localization and function. Mutations of this part led to aberrant motility, including a striking loss of directionality. As a high turnover of adhesion sites is a prerequisite for fast movement of sporozoites (Munter et al.), we hypothesize that coronin might be critical for adhesion site turnover. We are currently testing this hypothesis by biophysical measurements using optical tweezers, localization studies using an actin filament-binding chromobody and TIRF microscopy.

Bane et al., The actin filament-binding protein Coronin regulates motility in \emph{Plasmodium} sporozoites, PLOS pathogens, 2016

1738 The development and motility of \emph{T. brucei} in artificial human skin

Laura Hauf1, Christian Reuter1, Tim Krüger1, Florian Groeber-Becker2, Markus Engstler1 1) Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany; 2) Fraunhofer Institut für Silicatforschung ISC, Fraunhofer-Translationszentrum für Regenerative Therapien, Würzburg, Germany.

The unicellular parasite \emph{Trypanosoma brucei} is the causative agent of African trypanosomiasis and transmitted by the tsetse fly. Traditionally regarded as a primarily blood-dwelling parasite, the presence of \emph{T. brucei} populations within different host tissues has often been neglected. As a consequence, little is known about differentiation and lifestyle of the parasites outside the bloodstream. Of paramount interest in this context is the skin, as it is the site of transmission and therefore, the interface between host, parasite and vector.

In this project, the early events after parasite injection into the host skin were investigated. For this, an artificial human skin system was naturally infected through the vector, \emph{Glossina morsitans morsitans}. This experimental set-up allowed studies on tsetse fly injection path and depth, \emph{T. brucei} population kinetics in the skin, as well as parasite motility and developmental competence.

The population development of \emph{T. brucei} in human skin equivalents (SEs) was measured. The growth rate, cell cycle distribution and parasite stage transition was monitored over a period of 10 days post infection. Transmission of SE-derived trypanosomes to teneral tsetse flies revealed the full developmental competence of the parasites. Furthermore, the SEs mimic the physiological structure of native human skin; thus, they provide an expedient tool to investigate parasite motion inside the crowded fibroblast-collagen network. Parasite movement was tracked with single cell resolution using (stereo-)fluorescence microscopy.

1748 Ivermectin, a new old drug for Chagas disease?

\textbf{Maria Daniela Ruiz1, Agustina Claudio1, Luciana Larocca1, Verónica De Pino1, Carolina Carrillo1, Laura Fraccaroli1 1) ICT Milstein - CONICET - Buenos Aires, Argentina.}

Chagas disease is an endemic parasitosis originally from Latin America, caused by the protozoan \emph{Trypanosoma cruzi} (\emph{T. cruzi}). The current therapies (benznidazole - BZN and nifurtimox - NFX) are limited in efficacy and show multiple side effects. Thus, there is a need to identify new effective and specific trypanocidal strategies. Ivermectin (IVM) is a broad-spectrum antiparasitic drug of human and veterinary use. It is used for both ecto- and endo-parasite treatments and presents low toxicity in humans. These factors, along with its relative low cost, make IVM an interesting drug candidate for Chagas disease treatment. In previous studies, IVM has shown an effect against \emph{T. brucei} and \emph{Leishmania} in animal infection models.

Beginning our evaluation of IVM as a potential trypanocidal drug, the aim of this work was to characterize the effects of IVM on \emph{T. cruzi} epimastigotes and other trypanosomatids.

To approach this aim, we performed growth curves of epimastigotes of the Y-GFP strain in the presence of IVM (0 - 50 µM). The cultures were evaluated by cell counting in Neubauer chamber, optical density at 630 nm and MTT for 8 days. IVM dose dependently reduced the proliferation of the parasites. The relative density and the viability significantly decreased while duplication time increased, assessed at day 4 of culture. The EC50, also calculated at day 4 of culture, was 12.5 µM (10.8 - 14.5 µM). Recovery assays showed that IVM had trypanocidal or trypanostatic effects depending on the drug dose but not on the
exposure time, being trypanocidal at 100 μM IVM from 30 minutes up to 4 hours of exposure. The selectivity index was 2.4 (EC50 29.9 μM of IVM on Vero cell line).

To assess the interaction between IVM and BZN or NFX, epimastigotes were cultured in the presence of different combinations of those drugs according to a combination matrix and then relative density was analyzed by Combenefit and Compusyn softwares. This analysis showed mainly an additive effect with significant antagonism or synergism depending on the drug concentrations.

In related trypanosomatids, IVM affected the proliferation of *Phytomonas jima* and *Leishmania mexicana*, with an estimated EC50 of 5.5 μM and 11.7 μM respectively, while it did not affect *Critidia fasciculata*.

These results showed that IVM affects the proliferation and viability of *T. cruzi* epimastigotes and other trypanosomatids suggesting that it is a good candidate drug to continue the study in the Chagas disease context.

175B Exploring MKT1-PBP1-LSM12 complex as a novel stage-specific regulator of translation in *Plasmodium gametocyte* Elisha Mugo1, Roelof van Wyk1, Riëtte van Biljon2, Jandele Niemand1, Lyn-Marie Birkholz1 1) University of Pretoria, Department of Biochemistry, Genetics and Microbiology and the Institute for Sustainable Malaria Control, Pretoria, South Africa; 2) Pennsylvania State University, Department of Biochemistry and Molecular Biology and the Huck Center for Malaria Research Department of Chemistry, Pennsylvania, USA.

In preparation for transmission, *Plasmodium falciparum* gametocytes store hundreds of transcripts in translation silent RNA granules; this process is mediated by RNA binding proteins (RBPs). Only a few such proteins have been characterized in mature gametocytes, and it is not clear how mRNA dynamics observed in early gametocyte stages is regulated. Our goal is to characterize novel RNA binding proteins (RBPs) involved in the development of *P. falciparum* early gametocyte. After comparative transcriptome and proteome analysis of dataset obtained from *P. falciparum* as it commits to and develop through gametocytegenesis, we found MKT1, LSm12 and PBP1/Ataxin homolog protein to be among the twenty six high confidence candidate RBPs identified to be preferentially expressed in stage II/III early gametocytes. Interestingly, in the protozoan parasite *Trypanosoma brucei*, the essential MKT1-PBP1-LSM12 complex associate with actively translated mRNAs, localizes in stress granules and it has been implicated in cellular stress response and mRNA metabolism. Hence, it is possible that this complex can bind mRNAs in *P. falciparum* early gametocytes. Genetic and biochemical studies are currently underway to determine whether *Plasmodium PlmK1*, *PBP1* and PLSM12 homolog proteins either individually or as a complex influences early gametocyte mRNA translation and/or stability. This will provide novel insights into the post-transcriptional gene regulatory networks operating during *P. falciparum* sexual differentiation.

176A A novel quinoline-like action for the heme-artemisinin adduct metabolite Victoria Balta1, Wenchuan Ma2, Katy Newlin2, Ognjen Miljanić2, David Sullivan1, Peter Vekilov2, Jeffrey Rimer2 1) Johns Hopkins Bloomberg School of Public Health; 2) University of Houston.

Artemisinin’s primary mechanism of action involves the cleavage of the endoperoxide bridge by Fe(II) heme, resulting in free radical damage which interferes with numerous parasite functions. During activation by heme, a heme-artemisinin covalent adduct (H-ART) with inactivated artemisinin bound to the heme ring meso carbons can be formed. To date, the effect of exogenously applied H-ART on *P. falciparum* has not been characterized. Here, we show that exogenously applied H-ART and heme-artesunate adduct (H-ARS), synthesized in reducing conditions, inhibits both resistant Kelch13 mutant and sensitive *P. falciparum* in the 5 to 50 nM range in traditional 72 h. ICSO assays. Additionally, we show after exogenous H-ART and H-ARS pulses of trophozoites, both adducts are detected with hemozoin heme by mass spectrometry. Six hour ring or trophozoite stage pulse assays with 500 nM of H-ART, H-ARS, artesunate or chloroquine demonstrated that resistant Kelch13 mutant parasites had higher survival compared to sensitive NF54 and CamWT parasites in all drug groups. In ring stage pulses with H-ART, C580Y parasites had a survival rate of 70.5% whereas NF54 and CamWT parasites had survival rates of approximately 17%. Similarly, in ring stage pulses with H-ART, C580Y parasites had a survival rate of 20.6% whereas NF54 and CamWT parasites showed no survival. Additionally, we found that H-ARS inhibits resistant Kelch13 mutant parasites more than artesunate alone in ring stage assays, with a C580Y H-ARS survival rate of 20.6% and an ARS survival rate of 41.8%. As expected, PCR1-K767T mutant parasites showed greater survival after chloroquine pulse. In trophozoite stage pulse assays, both H-ART and H-ARS demonstrated near complete inhibition in all strains. These results suggest the irreversible addition of the adducts to the hemozoin crystal within the parasite. Given these results, we conclude that H-ART and H-ARS continue to inhibit *P. falciparum* growth after initial endoperoxide bridge activation through a quinoline like mechanism of action by inhibiting hemozoin crystal extension.

177B *Trypanosoma brucei* proteins: new allies in the fight against malaria? Adriana Temporão1, Margarida Sanches-Vaz1, Terry K. Smith2, Miguel Prudêncio1, Luis M. Figueiredo1 1) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; 2) Biomedical Sciences Research Complex, The North Haugh, The University, St. Andrews, Fife Scotland, United Kingdom.

Malaria, a disease caused by *Plasmodium* parasites, remains a major threat to global public health. In sub-Saharan Africa, regions where malaria is endemic overlap with those endemic for sleeping sickness, also called Human African Trypanosomiasis (HAT), a parasitic disease caused by *Trypanosoma brucei*. Malaria is the most common co-infection in patients with HAT [1]. Sanches-Vaz et al. have recently shown that an ongoing infection by *T. brucei* strongly impairs a secondary liver infection by *P. berghei* sporozoites in mice [2]. This inhibitory effect is phenocopied by the injection of a protein extract of bloodstream or procyclic forms of *T. brucei* 30 min prior to injection of *P. berghei* sporozoites. This finding paved the way to the current project, which aims at identifying the *T. brucei* protein(s) responsible for the inhibition of *Plasmodium* liver infection. We have shown that a subfraction containing proteins excreted *in vitro* by *T. brucei* is sufficient to elicit anti-*Plasmodium* activity observed, suggesting that the protein(s) responsible for inhibiting the *Plasmodium* liver stage are excreted by trypanosomes. Mass Spectrometry data showed that this fraction contains 31 proteins, most of which are involved in carbon metabolism. We will subsequently perform a genetic screen of the most promising *T. brucei* candidate genes, to identify the protein(s) involved in the observed phenotype. We expect that our results will identify a novel mechanism at play during a co-infection between two different parasite species, which may reveal novel strategies aimed at malaria control.

References


178B Grumpy IncRNA regulates life cycle progression of *Trypanosoma brucei* Fabien Guegan1, Fabio Bento2, Daniel Neves1, Mariana Sequeira1, Cedric Notredame3, Luisa Figueiredo1 1) Instituto de Medicina molecular – Joao Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Portugal, 2) : Institute of Molecular Biology and Institute of Developmental Biology and Neurobiology, Johannes Gutenberg Universität, 55128 Mainz, Germany ; 3) Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain.
Trypanosoma brucei causes African sleeping sickness, a fatal human disease. Its differentiation from replicative slender form into quiescent stumpy form promotes host survival and parasite transmission. Long noncoding RNAs (IncRNAs) are known to regulate cell differentiation. To determine whether IncRNAs are involved in parasite differentiation, we used RNAseq to survey the T. brucei IncRNA gene repertoire, identifying 1,428 previously uncharacterized IncRNA genes. We analysed grumpy, a IncRNA located immediately upstream of an RNA-binding protein that is a key differentiation regulator. Grumpy over-expression resulted in premature parasite differentiation into the quiescent stumpy form, and subsequent impairment of in vivo infection, decreasing parasite load in the mammalian host, and increasing host survival. Our analyses suggest Grumpy is one of many IncRNA that modulate parasite-host interactions, and IncRNA roles in cell differentiation are probably commonplace in T. brucei.

179B Addressing the VSG expression hierarchy in Trypanosoma brucei using single-cell RNA-sequencing

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African trypanosomes persist extracellularly in the bloodstream thanks to a remarkable capacity to exchange their variant surface glycoprotein (VSG) coat through a parasite-intrinsic process termed antigenic variation. Trypanosoma brucei chooses from an extensive repertoire of ~2600 different VSG genes during a switch event, most of which are organised in long subtelomeric arrays. A switch of VSG expression can be accomplished by either transcriptional or, more commonly, recombiniatorial mechanisms.

Previous studies have demonstrated the loosely ordered and preferential expression of certain subsets of VSGs in vitro and in vivo. To address if there is a hierarchical pattern in the choices made during VSG switching at the single cell level, we have developed a trypanosome-specific single-cell RNA sequencing method, based on molecular-crowding single-cell RNA-barcoding RNAseq (mcSCRBseq), that exploits the conserved spliced leader sequence present at the 5’ end of all mature protein-coding transcripts.

VSG switching occurs at a low frequency in laboratory-adapted cell lines, however it has been shown that a DNA double-strand break (DSB) within the active expression site can trigger a VSG switch. Taking advantage of this and the development of CRISPR/Cas9 tools in T. brucei, we have used CRISPR/Cas9 to introduce sequence-specific DSBs into the active VSG gene. Upon induction of Cas9 expression, we observe rapid formation of DSBs and highly reproducible kinetics of active VSG loss, with a population negative for the initial VSG (VSG2) emerging after 96 hours. Using our single-cell RNA-sequencing approach, we will observe the progression of VSG expression in single cells at regular time points following the induction of a DSB in the active VSG2 gene.

By overcoming the limitations of population-based approaches for monitoring VSG switches, our results will provide an exciting insight into the progression of T. brucei VSG expression during a switch event at the single-cell level.

180B The target of ergosterol biosynthesis inhibitors, CYP51 is dispensable for Trypanosoma cruzi epimastigote growth

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In addition to scavenging exogenous cholesterol, the parasitic kinetoplastid Trypanosoma cruzi is able to endogenously synthesize sterols. Similar to fungal species, T. cruzi synthesizes ergostane type sterols and is sensitive to a class of azole inhibitors of ergosterol biosynthesis that target the enzyme lanosterol 14α-demethylase (CYP51). Inhibition of CYP51 by azoles simultaneously results in loss of downstream sterols (e.g. ergosterol) and accumulation of 14-methylated sterol intermediates (e.g. lanosterol). In the related kinetoplastid parasite Leishmania donovani, CYP51 is essential, yet in Leishmania major, the cognate enzyme is dispensable for growth (but not heat resistance). The essentiality of CYP51 and the specific role of ergostane type sterol products in T. cruzi has not been established. To better understand the importance of this pathway we have disrupted the CYP51 gene in T. cruzi epimastigotes using a CRISPR/Cas9 and homology directed repair mediated strategy (ΔCYP51) along with genetic complementation. Disruption of CYP51 leads to accumulation of methylated sterol precursors (lanosterol and ebericol) and a concurrent absence of the final sterol product ergosterol, similar to treatment with azoles. While ΔCYP51 epimastigotes have slowed proliferation compared to WT parasites the enzyme is not required for growth. Consistent with the activity of the antifungal amphotericin B being mediated through ergosterol binding, ΔCYP51 epimastigotes are absolutely refractory to the permeabilizing effects of amphotericin B. Similar to L. major, the disruption of CYP51 in T. cruzi may sensitive parasites to additional external stressors. We are currently exploring the sensitivities of ΔCYP51 epimastigotes to stressors such as heat and pH. Additionally, CYP51 activity and endogenous sterol synthesis may fill different rolls in other life cycle stages of T. cruzi. Therefore, we will determine the ability of ΔCYP51 parasites to convert to metacyclic trypanosomes and infect mammalian cells.

181B Identification of clinically approved small molecules that inhibit growth and affect transcript levels of developmentally regulated genes in the African trypanosome

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Trypanosoma brucei are unicellular parasites endemic to Sub-Saharan Africa that cause fatal disease in humans and animals. Infection with these parasites is caused by the bite of the tsetse fly vector, and parasites living extracellularly in the blood of infected animals evade the host immune system through antigenic variation. Existing drugs for Human and Animal African Trypanosomiasis are difficult to administer and can have serious side effects. Resistance to some drugs is also increasing, creating an urgent need for alternative trypanosomiasis therapeutics. We screened a library of 1,585 U.S. or foreign-approved drugs and identified 154 compounds that inhibit trypanosome growth. As all of these compounds have already undergone testing for human toxicity, they represent good candidates for repurposing as trypanosome therapeutics. In addition to identifying drugs that inhibit trypanosome growth, we wished to identify small molecules that can induce bloodstream form parasites to differentiate into forms adapted for the insect vector. These insect stage parasites lack the immune evasion mechanisms prevalent in bloodstream forms, making them vulnerable to the host immune system. To identify drugs that increase transcript levels of an invariant, insect-stage specific surface protein called procyclin, we engineered bloodstream reporter parasites that express Green Fluorescent Protein (GFP) following induction or stabilization of the procyclin transcript. Using these bloodstream reporter strains in combination with automated flow cytometry, we identified efornithine, spironolactone, and phenothiazine as small molecules that increase abundance of procyclin transcript. Both efornithine and spironolactone also affect transcript levels for a subset of developmentally associated genes. While we failed to identify compounds that increase levels of procyclin protein on the cell surface, this study is proof of principle that these fluorescent reporter parasites represent a useful tool for future small molecule or genetic screens aimed at identifying molecules or processes that initiate remodeling of the parasite surface during life cycle stage transitions.

182B Identification and characterization of iron transporters in the glycosomes of Leishmania

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The protozoan parasites of the genus Leishmania are responsible for the diseases known as leishmaniasis that affect millions of human worldwide. These parasites life cycle includes invertebrate and vertebrate hosts. In the vertebrate hosts, they are able to survive and replicate in macrophages, despite the defense arsenal of these cells. One of the critical conditions found by Leishmania in the macrophage is the lack of various nutrients, among them iron, which is a
cofactor of several enzymes essential for the parasite. The identification and study of parasite genes involved in iron metabolism and transport revealed that the availability of iron plays a central role in virulence. Besides this, it was also shown that iron deprivation induces the expression of a series of genes whose function is still unknown. Among these, some essential genes encoding proteins addressed to the glycosomes, which are trypanosomatids unique organelles. These findings indicate that the transport of iron to the glycosome, which has not been explored so far, is also an interesting target for identification and study. Therefore, our work aims to identify and characterize genes involved in the transport of iron into the Leishmania glycosome. For this, we initially performed the in silico analysis of 576 Leishmania transcripts significantly modulated by iron deprivation looking for those that contained predicted glycosomal addressing signals (PTS1 or PTS2) and predicted transmembrane domains, and we found 11 putative genes. To confirm the subcellular localization of the proteins encoded by these 11 genes, we cloned the genes’ ORFs upstream or downstream of the GFP coding sequence into the pXG-GFP+ and pXG-GFP+Leishmania overexpressing plasmids. Thereby, we will confirm the subcellular localization of these proteins in L. amazonensis and start the construction of the plasmids to knockout the glycosomal proteins. The knockout of the genes encoding glycosomal proteins will allow the elucidation of the role of these genes, and particularly the role of glycosomal iron, in L. amazonensis iron metabolism, replication and virulence. Our perspective is to identify new proteins present only in the parasite and essential for their survival in the host, as potential targets for the development of new drugs for leishmaniasis treatment.

183B Trypanosome RNA Editing Substrate Binding Complex integrity and function depends on the upstream action of RESC10 Ashutosh Dubey1, Brianna Tylec1, Natalie Mcdams1, Joseph Smith1, Katherine Sortino 1, Laurie Read1 1) University at Buffalo.

Uridine insertion/deletion editing of mitochondrial mRNA is a characteristic feature of kinetoplastid parasites including Trypanosoma brucei. Editing is directed by trans-acting gRNAs and catalyzed by related RNA Editing Core Complexes (RECCs). A second complex, the non-catalytic RNA Editing Substrate Binding Complex (RESC), coordinates interactions between RECC, gRNA, and mRNA by ill-defined mechanisms. RESC contains two sub-modules: REMC (RNA Editing Mediator Complex) and GRBC (Guide RNA Binding Complex). The goal of this study is to determine the function of one of the less well studied RESC proteins, RESC10 (Tb927.7.800). We show that RESC10 is an essential, relatively low abundance, RNA binding protein that exhibits RNA-dependent and RNA-independent interactions with multiple RESC proteins. Surprisingly, however, its in vivo interaction with RESC13 (aka, TbrG622) is minimal. RESC10 depletion leads to extensive RESC disorganization, including disruption of intra-GRBC protein-protein interactions, suggesting this protein is not a dedicated REMC module factor as previously reported. RNA immunoprecipitations in induced and induced RESC10 RNAi cells show that mRNAs are depleted from GRBC and accumulate on REMC upon RESC10 knockdown. RESC10 is essential for procryst form T. brucei growth and for editing of both pan-edited and moderately edited mRNAs. We observe a 70-90% decrease in all edited mRNAs tested, constituting one of the most dramatic effects to date of RESC protein depletion. Analysis of mitochondrial RNAs at single nucleotide resolution reveals transcript-specific effects: RESC10 dramatically impacts editing progression of pan-edited RPS12 mRNA, but is critical for initiation of those mRNAs with internally initiating gRNAs (ND7-5’, Cyb, MURF2), pointing to distinct mechanisms for editing initiation between these RNA classes. Correlations between sites at which editing pauses in RESC10 depleted cells and those in previously reported RESC knockdowns suggest that RESC is particularly important in promoting transitions between U insertion and U deletion RECCs. Together, our data identify RESC10 as a protein critical for proper protein-protein and protein-RNA interactions within RESC. Our findings are consistent with a model in which RESC10 interacts with other RESC proteins early in their assembly, and possibly transiently, to promote RESC integrity.

184B Investigation of Puf2-Mediated Post-Transcriptional Regulation in Sporozoites Michael Walker1, Tyler Earp1, Scott Lindner1 1) Department of Biochemistry and Molecular Biology, Center for Malaria Research, Pennsylvania State University, University Park, PA.

Puf (Pumilio and fem-3 binding factor) proteins are RNA-binding proteins conserved throughout eukaryota. In Plasmodium, Puf proteins have been shown to have distinct roles in the transcription stages of the parasite. Specifically, in Plasmodium yoelii sporozoites, PyPuf2 disruption leads to a decrease in infectivity during prolonged salivary gland residence. This severe phenotype coincides with a massive dysregulation of transcripts that are important for the future life cycle stages, indicating that Puf2 may play a role in the preservation of transcripts important for the infectivity of the parasite. However, the transcripts that are directly regulated by PyPuf2, as well as how they are regulated, remain nebulous. In order to determine the method of regulation, we are interrogating the one transcript that is most clearly affected by PUF2: uis4 (up-regulated in infective sporozoites). In previous studies with Plasmodium berghei, it was demonstrated that while the 5’ and 3’ UTRs were dispensable for translational repression, the ORF of uis4 was required for regulation by Puf2. Here we have used the CRISPR-RGR genome editing system with Plasmodium yoelii to change portions of the pyuis4 coding sequence and to simultaneously append a C-terminal GFP tag, which permits detection of PyUIS4:GFP protein expression through fluorescence-based approaches. In order to determine which portion(s) of the uis4 ORF is necessary for translational repression, we have recodized individual thirds (~200bp) of the ORF, or have mutated the two bioinformatically predictable Puf Binding Elements (PBE). Contrary to our expectations, we found that both PBEs are dispensable and that translational repression still occurs in their absence. Finally, to experimentally determine the set of transcripts that are bound by PyPuf2, we are performing complementary RNA Immunoprecipitation (RIP) and RNA labeling assays in salivary gland sporozoites. These datasets will identify transcripts that are regulated directly by PyPuf2 during mosquito-to-mammal transmission. The combination of these projects would certainly broaden our understanding of Puf2’s role in the maintenance of sporozoite infectivity during salivary gland residence.

185B Unraveling the role of TcVps34-Vps15 complex as a modulator of autophagy and metacyclogenesis in Trypanosoma cruzi Alejandro Schojiett1, Tamara Sternlieb1, Guillermo Alonso1 1) Institute for Research on Genetic Engineering and Molecular Biology (INGEBI-CONICET-UBA).

Chagas disease affects millions of Latin America individuals; however, due to the immigration of infected people, this illness has emerged in non-endemic countries. Chemotherapy is based on two nitrocompounds, benznidazole and nifurtimox, that show limited effectiveness in chronic phase, culminating in severe collateral effects. Thus, effective therapy against the chronic form of Chagas disease has yet to be discovered and developed. Autophagy is a conserved mammal transmission. The combination of these projects would certainly broaden our understanding of Puf2’s role in the maintenance of sporozoite infectivity during salivary gland residence.

186B Trypanosoma brucei DNA Polymerase θ is Essential for Telomere Integrity and Suppresses VSG Switching Maiko Tomin1, M A G Rabban1, Bibo Li1 1) Cleveland State University.

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Trypanosoma brucei is a protozoan parasite that causes human African trypanosomiasis. While proliferating in the extracellular spaces in its mammalian host, T. brucei regularly switches its major surface antigen, VSG, to evade the host immune response. T. brucei has a large VSG gene pool, and all VSG genes are located at subtelomeric loci. However, VSG is expressed exclusively from one of thirteen subtelomeric VSG expression sites (EJs) in a strictly monoallelic expression fashion. Our lab has shown that many telomere proteins play critical roles in VSG regulation. Although DNA double strand breaks (DSBs) are frequently repaired by homologous recombination (HR) or Non-Homologous-End Joining (NHEJ) in many organisms, DNA ligase 4 that is essential for NHEJ is absent in the T. brucei genome, and no NHEJ events have been described in this parasite. In contrast, HR has been shown to play a major role in VSG switching. Recently, Microhomology-Mediated end Joining (MMEJ) events have been described in T. brucei, although whether it is normally involved in VSG switching is not clear. We recently identified T. brucei DNA Polymerase θ as a telomere protein and is essential for cell proliferation. In mammalian and yeast cells, DNA Pol θ is a key player in MMEJ and is important for DNA damage repair. We found that T. brucei cells lacking DNA Pol θ are more sensitive to DNA damaging agents and display increased amount of DNA damage, particularly at the telomere. Interestingly, depletion of DNA Pol θ also results in VSG derepression and increased VSG switching frequency. Additional observations further indicate that T. brucei DNA Pol θ plays a critical role in telomere integrity.

187B Distribution and phylogenetic diversity of Anopheles species in main malaria endemic areas of Honduras Denis Escobar1, Krisnaya Ascencio1, Andrés Ortiz1, Adalid Palma1, Gustavo Fontecha1 1) National Autonomous University of Honduras.

Malaria is still an important health issue worldwide, Anopheles mosquitoes transmit this disease in several countries in the tropics. More than 500 Anopheles species have been described worldwide, and more than 30 are considered a public health problem. In Honduras, information on the distribution of Anopheles spp. and its genetic diversity is scarce. This study aimed to describe the distribution and genetic diversity of Anopheles mosquitoes in Honduras.

Entomological captures were done in 8 locations in 5 malaria endemic departments during 2019. Two collection methods were used. Adult anophelines were captured outdoors using CDC light traps and by aspiration of mosquitoes at nest. Morphological identification was performed using classic taxonomic keys. Genetic analyses included the sequencing of a partial region of the cytochrome c oxidase 1 gene (cox1) and the ribosomal internal transcribed spacer 2 (ITS2).

A total of 1320 anophelines were collected and identified through morphological keys. Seven Anopheles species were identified. Anopheles albimanus was the most widespread and abundant species described (74.02%). To confirm the morphological identification of the specimens, 175 and 122 sequences were obtained for cox1 and ITS2, respectively. Both markers confirmed the morphological identification. cox1 showed a greater nucleotide diversity than ITS2 in all species. High genetic diversity was observed within the populations of An. albimanus while An. darlingi proved to be a highly homogeneous population. Phylogenetic analyses revealed clustering patterns in An. darlingi and An. neivai in relation to specimens from South America. New sequences data for An. crucians, An. vestitiennis and An. neivai are reported in this study.

Here we report the distribution and genetic diversity of seven Anopheles species in endemic areas of malaria transmission in Honduras. According to our results, both taxonomic and molecular approaches are useful tools in the identification of anopheline mosquitoes. However, both molecular markers differ in their ability to detect intraspecific genetic diversity. These results provide supporting data for a better understanding of the distribution of malaria vectors in Honduras.

1888 Structural and phylogenetic analysis of Trypanosoma cruzi’s major intrinsic proteins (MIP) Fiorella Tesan1,2, Gerardo Zerbetto de Palma1,2,3, Luciano Armenta1, Ramiro Lorenzo4, Ari Zeida5, Federico Penas6, Nora Goren6, Romina Fox4, Karina Alvea1,2,1 Catedra de Fisica, FYFB (Universidad de Buenos Aires), CABA, Argentina; 2) IQUIFIB (UBA/CONICET), CABA, Argentina; 3) Instituto de Biotecnología, Universidad Nacional de Hurlingham, Villa Tesei, Argentina; 4) CIVETAN (CONICET), Tandil, Argentina; 5) Departamento de Bioquímica y Centro de Investigaciones Biomédicas (Cebinio), Facultad de Medicina, Universidad de la República, Uruguay; 6) Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (UBA/CONICET), CABA, Argentina.

The rise in genome and transcriptome sequencing revealed a great diversity of aquaglyceroporins (Glp) and aquaporins (Aqp) (MIP, Major Intrinsic Protein superfamily) throughout the three kingdoms of life: Archaea, Bacteria and Eukarya. Some of these channels, first identified as water transporters, also transport solutes such as glycerol, hydrogen peroxide, etc. Also, some of these channels are involved in the internalization of antiparasitic drugs in kinetoplastids: pentamidine for Trypanosoma brucei and trivalent antimony for Leishmania. Trypanosoma cruzi genome encode four Aqps (TcAqps) and only one of them was characterized (transport and localization). Thus, the aim of this work is to characterize the structure of TcAqps to gain knowledge on their structure-function particularities and to study their evolutionary context.

Aqp compatible sequences were searched for within Trypripdb and NCBI databases (annotated as Aqp or resulted from tblastn search). Multiple sequence alignments (MAFFT, v7) were performed to build phylogenetic trees (Maximum Likelihood). Synteny studies were performed for those Trypripdb retrieved sequences. 3D models were built for the four TcAqps (Brener NEL stain) by ab initio methods (Rosetta, iTasser) and pores were characterized based on molecular dynamic analyses (AMBER14SB and LIPID14 force field, Hole).

Conserved AQP like tetrameric structures were found in all constructed 3D models. MIP asymmetry was found to be the distinctive feature between american and african trypanosomas (not only T. brucei and T. cruzi) with the exception of T. grayi. For most of the other Trypanosomatida genera we found both Aqp and Glp MIPs. Aqps coding regions were lost in african trypanosomes within syntenic blocks. From the key MIP residues analysis, Trypanosomatida Aqps display increased amount of DNA damage, particularly at the telomere. Interestingly, depletion of DNA Pol θ also results in VSG derepression and increased VSG switching frequency. Additional observations further indicate that T. brucei DNA Pol θ plays a critical role in telomere integrity.

1989 Cyclic AMP binding proteins in Trypanosoma cruzi José Escalona1, Guillermo Di Mario2, Gabriel Ferri2, Martín Edreira1,2,3 1) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina; 2) CONICET-Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUBICEN), Ciudad de Buenos Aires, Argentina; 3) Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

Cyclic AMP signaling have shown to be involved in Trypanosoma cruzi biology. However, little is known about the downstream effectors in this pathway in the parasite. Previous reports showed that at the genomic level this parasite has several proteins that possess cyclic nucleotide binding domain (CNB), such as Protein kinase A (PKA). Although, it has also been published that, unlike its mammalian counterpart, the trypanosomatid PKA does not bind cAMP. In order to increase the knowledge about the cAMP mediated pathway in T. cruzi, we have cloned and biochemically characterized several CAMP binding proteins in T. cruzi. At the bioinformatic level, several proteins were found in the parasite’s genome. Six candidates, TcCLB.418221.20, TcCLB.504153.20, TcCLB.504449.30, TcCLB.508523.80, TcCLB.510691.30 and TcCLB.508273.30 were selected to perform docking models to evaluate cAMP binding capability in silico. These candidates then were cloned and expressed with his-tag or GST-tag in E. coli. Bacterially expressed proteins and a CAMP-agarose resin were used in pull down and displacement experiments, in order to confirm cAMP binding. Interaction assays were then performed using trypomastigote lysates obtained from infected vero cell cultures. The eluted proteins were analyzed by mass spectrometry obtaining potential partners candidates, that will be further studied.

1908 Role of a TgVps15 in the development of Toxoplasma gondii Rahul Singh Rawat1, Priyanka Bansal1 1) National Institute of Immunology, New Delhi, India.
Role and regulation of 3'-phosphorylated phosphoinositide (PI3P) in the development of apicomplexan parasites such as Plasmodium and Toxoplasma spp. is poorly understood. A single phosphoinositol-3 kinase (PI3K) catalyses the formation of PI3P by phosphorylating phosphoinositol (PI) in these parasites. PI3P plays a critical role in apicoplast biogenesis in Toxoplasma. In the present study, the role of a putative Vacuolar Protein Sorting 15 protein (Vps15) was studied in Toxoplasma gondii. Vps15 is a regulator of Vps34 (class III PI3Ks) conserved in most eukaryotes and regulates its activity. A Vps15 like protein (TgVps15) is present in Toxoplasma gondii. It possesses a protein kinase domain like yeast and mammalian homologs; but several key residues critical for protein kinase activity and subdomains are absent. Surprisingly, kinase assays performed with recombinant kinase domain of TgVps15 revealed that it autophosphorylates. Since, TgVps15 is essential for parasite survival, conditional knockdown approaches were employed to deplete TgVps15 in Toxoplasma gondii. TgVps15 was found to be involved in PI3P generation as its depletion impaired the localization of a PI3P-reporter, which is targeted to the apicoplast. TgVps15 regulates parasite division and its depletion resulted in “delayed death”. Phenotypic characterization revealed that it regulates apicoplast biogenesis. It may achieve this via autophagy related protein 8 (ATG8), which has previously been implicated in apicoplast inheritance. Collectively, these studies shed light on novel pathway regulated by TgVps15 in T. gondii and possibly in other Apicomplexans.

191B Molecular characterization of acute virulence loss in heme-deficient Toxoplasma gondii Amy Bergmann1,2, Zhicheng Dou1,2 1) Department of Biological Sciences, Clemson University, Clemson SC; 2) Eukaryotic Pathogens Innovation Center (EPIC), Clemson University, Clemson, SC.

Toxoplasma gondii is an apicomplexan parasite with an active de novo heme biosynthesis pathway. Our latest publication showed that T. gondii requires this pathway for infections. The heme deprivation in the parasites causes the loss of their acute virulence in a murine model. Previous studies have shown that Toxoplasma uses several strategies to evade the host’s innate immunity, such as repression of nitric oxide production and resistance of the attack from the host’s immunity-related GTPase (IRG) within activated macrophages. In this study, we determined the extent to which the heme-deficient parasites manipulate these two aspects of host immunity evasion. Two heme-deficient parasites, coproporphyrinogen III oxidase (TgPPO)- and protoporphyrinogen oxidase (TgPPO)-lacking mutants, named Δprop and Δprop, respectively, were included in this work for independent evaluation. Our data showed that both Δprop and Δprop mutants exhibit a lack of ability to inhibit NO production in IFN-γ and lipopolysaccharide (LPS)-activated RAW cells. In addition, the expression of two Toxoplasma rhoptry proteins used in preventing an attack from the host’s effectors, TgROP5 and TgROP18, were reduced in heme-deficient parasites. These findings may provide an explanation of why the heme-deficient mutants cannot efficiently circumvent the host’s immunity attack. Previous literature reported that a Toxoplasma dense granule protein, named TgVps15 Inhibitor of STAT1-dependent Transcription (TgIST), is involved in blocking IFN-γ dependent transcription, which further helps parasite survival in IFN-γ activated immune cells. Currently, we are investigating if the expression and translocation of TgIST are altered in heme-deficient parasites. Overall, our findings will enhance the understanding of how heme abundance in the parasites mediates the pathogenesis of toxoplasmosis.

1928 TgCEP250L1 is essential for cell division in Toxoplasma gondii Ramirio Tomasina1, Mathieu Gissot3, Maria E Francia,1,2) 1) Institut Pasteur de Montevideo, Montevideo, Uruguay.; 2) Department of Parasitology and Mycology. School of Medicine. Universidad de la Republica. Uruguay; 3) Institut Pasteur de Lille, Lille, France.

Cell division in Apicomplexa is essential for parasite survival within the host. The molecular mechanisms governing the process are divergent from the ones used by our cells. In particular, apicomplexans use physical tethers to the centrosome, the main microtubule organizing center of the cell, instead of soluble factors, to coordinate the progression of different events required to produce new, infection competent, parasites. Ultrastructural observation and molecular biology experiments have shown that the organization and composition of the Apicomplexan centrosome is highly divergent, making it an attractive, yet relatively unexplored, potential drug target. The centrosome in Toxoplasma gondii is constituted by two core domains; an outer core, distal from the nucleus, and the inner core (proximal to the nucleus) (Suvorova, et al, 2015). The outer core is thought to coordinate the assembly of the daughter cell. The inner core is thought to control event related to the translocation of the nucleus. The latter, however, remains to be demonstrated. Here we generate a mutant strain whereby TgCEP250L1, an inner core protein, is conditionally degraded. The iKO of TgCep250L1 parasites fail to form plasmodia, suggesting that TgCep250L1 is essential for parasite survival. Mutant phenotype analysis by immunofluorescence assays shows that, in the absence of TgCep250L1, parasite exhibit nuclear segregation defects, while normally forming daughter cells. Moreover, the centrosome disconnects from the nucleus, suggesting TgCep250L1 plays an essential role in coordinating nuclear and centrosome segregation and daughter cell formation. Overall, our results support a role for the inner core in nuclear segregation. We are currently exploring the importance of TgCep250L1 in nuclear homeostasis and mitotic spindle assembly.

1938 Crystal violet structural analogues as candidates for drug repurposing in trypanosomatid diseases Chantal Reigado1, Nicky Didwania2, Melissa Saye1, Nahid Ali2, Claudio A Pereira1 1) Instituto de Investigaciones Médicas (IDIM, UBA-CONICET), CABA, Argentina; 2) CSIR-Indian Institute of Chemical Biology, Kolkata, India.

Trypanosoma cruzi, the etiological agent of Chagas disease, has a metabolism largely based on the consumption of glucose and proline. Proline is also involved in differentiation processes, cellular invasion and resistance to different stresses. Up to date, the permease TcAAP0069 is the only proline transporter identified in T. cruzi and we have reported its importance on parasite survival. Interestingly, the AAP transporters family is present in all trypanosomatid parasites and is low represented in mammals. Crystal violet (CV) was used for several years as a blood additive for prevention of transfusion-transmitted Chagas disease. Recently, we have validated TcAAP0069 as one of the targets of CV and then we applied a virtual screening approach in order to identify drugs already approved in humans that might have similar effects to CV. The antihistamines loratadine (LTD) and cyproheptadine (CPH), and the antibiotic clofazimine (CFZ) were able to inhibit proline transport and had trypanocidal effect with IC50 values (the concentration that kills 50% of the parasites) between 1 and 13 µM in trypanomastigotes and amastigotes.

We also evaluated the in vitro antiprotozoal effect of these drugs in T. brucei and L. donovani, the causative agents of human African trypanosomiasis and visceral leishmaniasis, respectively. The CV structural analogues presented IC50 values between 1 and 31 µM in procyclic trypomastigotes of T. brucei. These compounds also had activity against L. donovani promastigotes with IC50 values between 2 and 44 µM. In addition, preliminary tests with cationic liposomal formulations of the drugs on this parasite showed that drug-loaded liposomes presented higher leishmanicidal activity than free compounds. Since proline permease TcAAP0069 has orthologous genes in Leishmania and T. brucei, LdAAP24 and TbaAT6, these compounds will be evaluated as inhibitors of the proline transport on both parasites as possible mechanism of action of such drugs.

The strategy herein applied, based on the screening of approved compounds used to treat other pathologies, is known as drug repurposing. One of the main advantages of computer-aided drug repurposing is that reduces the time and the economic cost of implementation of new therapeutic alternatives, which is especially important in neglected diseases.

1948 Cathespin C-like protease 1 post-translationally modifies Toxoplasma gondii secretory proteins for optimal invasion and egress L. Brock Thornton1,2, Chiara Michelli1,2, Melanie Key1,2, Andrew J. Stasic3, Katherine Floyd1, Silvia N. J. Moreno3, Zhicheng Dou1,2 1) Department of Biological Sciences, Clemson University, Clemson, SC; 2) Eukaryotic Pathogens Innovation Center (EPIC), Clemson University, Clemson, SC; 3) Department of Cellular Biology, University of Georgia, Athens, GA.
The obligate intracellular protozoan parasite Toxoplasma gondii infects roughly one-third of the global human population and is of particular concern to immunocompromised individuals. In order to establish infection and survive intracellularly, T. gondii parasites must synthesize, process, and secrete specialized effector proteins. For example, Toxoplasma utilizes microneme proteins for attachment and invasion of host cells. Previous work has shown that some of these micronemal invasion effectors are proteolytically processed by an aspartyl protease, named TgASP3, within a post-Golgi compartment. The endolysosomal system in T. gondii is an additional hub for protein trafficking and processing. This pathway includes the lysosome-like vacuolar compartment/plant-like vacuole (VAC/PLV) as well as the endosome-like compartment (ELC). Housed within these organelles are cathepsins proteases which have also been shown to play an important role in the proteolytic maturation of micronemal invasion effectors. For example, previous studies have shown that the VAC/PLV-localized, cathepsin L-like protease (TgCLP) matures TgMIC3 and TgMIC2-associated protein (TgMICAP). Additionally, Toxoplasma expresses a cathepsin B-like protease and two cathepsin C-like proteases during acute infection. One cathepsin C-like protease 1 (TgCPC1) was previously identified as a dense granule protein. In this study, through the use of two independent strategies of epitope tagging TgCPC1 at different locations, we found that this protease largely localized to the ELC but could also be detected within the VAC/PLV. Furthermore, the TgCPC1 gene was genetically ablated and the resulting Δcpc1 mutant displayed defects in multiple steps within the lytic cycle, specifically invasion, egress, and migration. Further investigation revealed that Δcpc1 displayed defective secretion and trimming of several microneme proteins, most notably TgM2AP, TgMIC5, and perforin-like protein (TgPLP1), while protein trafficking was unaltered. Interestingly, the processing of the subtilisin protease (TgSUB1) was completely blocked within Δcpc1. Similarly, defective trimming of TgM2AP and TgSUB1 was also observed in parasites treated with an inhibitor targeting the Plasmodium ortholog of TgCPC1, further suggesting that TgCPC1 plays a significant role in the processing of micronemal proteins. Taken together, these results indicate that TgCPC1 plays a significant role in the processing of secreted effector proteins which are ultimately crucial for successful invasion and egress of host cells.

1958 Insights on new Phosphatidylinositol transfer proteins (PITPs) in Toxoplasma gondii Angela Arabiotortre1, Aby Grabon1, Vyta Bankaitis1,2,3
Department of Molecular and Cellular Medicine, Texas A&M University (PITP); 2) Department of Biochemistry and Biophysics, Texas A&M University, College Station; 3) Department of Chemistry, Texas A&M University, College Station.

T. gondii infects most species of warm-blooded animals, including humans. It forms tissue cysts in diverse organs that remain throughout the life of the host without causing illness when faced to a competent immune system. However, Immunocompromised patients and women during pregnancy experience severe symptoms leading to death. T. gondii is an Apicomplexan parasite that utilizes a unique set of specialized late secretory organelles - micronemes, rhoptries, and dense granules - to secrete invading effectors into the host cytoplasm. The uniqueness of this secretory system is a promising target for therapeutic intervention. Phosphoinositides (PIPs), the phosphorylated forms of phosphatidylinositol (PtdIns), function as critical intracellular chemical signals in eukaryote, specifically in the secretory pathway. For instance, phosphatidylinositol-4-phosphate (PtdIns[4]P) in yeast is an essential potentiator of membrane trafficking in the trans-Golgi network (TGN)/endosomal pathway. Additionally, it has been determined that the yeast PITP Sec14 exerts instructive regulation of PtdIns 4-OH kinase activities and channel Golgi PtdIns[4]P pool production required for efficient membrane trafficking from the TGN. To understand whether PtdIns[4]P pools in T. gondii’s secretory membranes might be subject to similar regulation, we are focused in describing the cohort of PITPs encoded by the parasite genome. Quite recently our group has identified a new PITP in T. gondii, termed PITP Multidomain Protein (PIMP). Strikingly, the Pleckstrin homology (PH) domain of this protein is capable of sensing PtdIns(4)P within the parasite. This pools appear to be involved in trafficking of dense granule proteins (GRA3/2) through post-Golesi endosome-like compartments. This was shown by observing accumulation of GRA3/2-RFP fused protein in the cytoplasm (large puncta pattern) of ~45% of parasite population when accessibility to PtdIns(4)P pools was compromised. Other PIP pools were also compromised (PtdIns(3,4)P and PtdIns(4,5)P) revealing that PtdIns(4)P pools exclusively affect GRA3/2 trafficking. Formation of the large puncta pattern might be due to gathering of dense granules, possibly trapped in a step of the secretion process. This was shown in a chasing experiments using Cycloheximide (CHX). Furthermore, other PITP prospective proteins have been identified as they appear to contain a Sec14-like domain. These proteins are SfhA, SfhB, SfhC and SfhD (SFH: Sec14 homology family). Curiously the Sec14-like domain in SfhA shares an important sequence similarity to Yeast Sfh1, and its ability to transfer PtdIns appears to be lacked. Interestingly, SfhA has shown to bind in small degree squalane, which might suggest its involvement in the metabolism of cholesterol, an essential lipid that is sequestered from the host cell.

196C An insight into the penetration process of a tsetse bite Stephan Löwe1, Dennis Petersen2, Georg Krohne1, Stanislav Gorb2, Markus Engstler1
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The bloodsucking tsetse fly (Glossina spec.) transmits African trypanosomes between a variety of hosts. The success of parasite transmission highly depends on the efficiency of the host skin penetration through specialized mouthparts of the fly. Though studies on the morphology of the proboscis of Glossina pallidipes have been conducted [1], it remains unclear which mechanics and forces govern the penetration process of the tsetse fly. Our current work wants to solve this issue.

First, we used scanning electron microscopy to describe external and internal structures of the tsetse mouthparts. The feeding process occurs by the protrusion of the proboscis through a substrate. In order to imitate vertebrate skin conditions we used heated silicon-based models.

The proboscis is a functional morphological unit in which three mouthparts: labrum, labium and hypopharynx are fused to a long and slender tube. The anterior part of the proboscis is characterized by the labellum that bears various teeth-like structures. These are responsible for piercing through different range of millinewtons. Our results further reveal that, in contrast to the general perception, not only the protrusion but the forcefully repeated retraction is the key process for penetration.

In order to understand this issue, we performed a penetration assay and our results demonstrate a significant increase of “flagellar swellings” formed in response to attachment to host cell in both parasites, suggesting a role of this structures in this process. It is currently known that the flagellar membrane is a


197C Ultrastructural and functional analysis of extra-axonomal structures in trichomonads veronica cocerests1, Antonio Pereira-Neves 2, Natalia de Miguel1, Abigail Miranda-Magalhães4, Thiago André Santos de Andrade5, Lucrecia Iriarte6 1) Instituto Tecnológico Chascomús (INTECH); 2) Instituto Aggeu Magalhães, Fiocruz, Recife, Pernambuco; 3) Instituto Tecnológico Chascomús (INTECH); 4) Instituto Aggeu Magalhães, Fiocruz, Recife, Pernambuco; 5) Instituto Aggeu Magalhães, Fiocruz, Recife, Pernambuco; 6) Instituto Tecnológico Chascomús (INTECH).

Trichomonas vaginalis and Tritrichomonas foetus are flagellated extracellular parasites that inhabit the urogenital tract of human and cattle, respectively. T. vaginalis causes the most common non-viral sexually disease worldwide; while T. foetus is responsible of reproductive disorders in cattle of different regions of the world. Given it are extracellular parasites, attachment to host cells is one of the key processes for the development of infection. At this sense, although the motility of flagella has been described as essential in host invasion in other parasites, it molecular organization and biological function remain poorly understood in Trichomonads. Based on this, we analyzed the flagella structure of wild-type parasites (T. foetus/T. vaginalis) by SEM/TEM and demonstrated the existence of membrane expansions with extra material intrflagellar or “flagellar swellings” (in the tip and along of flagella) during host–parasite and parasite-parasite interactions. Then, we performed an attachment assay and our results demonstrate a significant increase of “flagellar swellings” formed in response to attachment to host cell in both parasites, suggesting a role of this structures in this process. It is currently known that the flagellar membrane is a
highly specialized subdomain of the surface membrane, and flagellar membrane proteins are likely to be critical components for all the biologically important roles of flagella. The ESCRT protein complex comprises several sub complexes (ESCRT-0/ESCRT-III) involved in membrane fission events that could be important for ciliary or flagellar membrane dynamics. Being VPS32 the most abundant protein of the ESCRT-III, we overexpress TTVPS32-HA and TVVPS32-HA in both parasites using empty vector as a control (EpNEO). We demonstrated that TTVPS32-HA and TVVPS32-HA are localized in “flagellar swellings” plasma membrane by immunogold electron microscopy. Interestingly, a significant increase of flagellar swellings, 15% and 18% were found in TTVPS32-HA and TVVPS32-HA transfected parasites, respectively; compared to 5% of flagellar swellings observed in EpNeo and wild types parasites; suggesting a possible role of this protein in flagellar swellings formation. In summary, our findings suggest that there might be a relationship between VPS32 protein and dynamics events that occurs in the plasma membrane such vesicles scission and flagellar membrane remodeling events during attachment, property critical for the pathogenesis.

198C Nested PCR for the detection of Taenia solium DNA in stool samples Carlos Franco-Muñoz1, Sofia Duque 1, Adriana Arevalo 1 1) Instituto Nacional de Salud.

The traditional parasitological method to diagnose taeniasis is the microscopic observation of eggs in stool samples. However, this method does not allow differentiation between Taenia saginata and Taenia solium. This aim of this study was to achieve the detection of T. solium DNA by polymerase chain reaction (PCR) and to evaluate the cross-reaction with other species of the genus Taenia and other intestinal parasites. DNA was extracted from adult T. solium cestodes by cryolysis in liquid nitrogen and with the DNA stool extraction kit from Qiagen. The detection limit of the test was evaluated by DNA dilutions in water and in stool samples. DNA was extracted from proglottids of T. saginata and T. crassiceps and from stool samples containing other intestinal parasites using ethanol treatment, alkaline lysis, and the DNA stool extraction kit. Nested PCR was used to amplify a previously described fragment of the Tso31 gene, and the PCR products were analyzed by electrophoresis in 2% agarose gels followed by staining with GelRed. The nested PCR of the Tso31 gene allowed the detection of T. solium DNA in stool samples with a detection limit of 20 pg of parasite DNA. PCR showed no cross-reaction with T. saginata, T. crassiceps, or other intestinal parasites of public health importance in Colombia.

199C Effect of lysine acetylation on the regulation of Trypanosoma brucei glycosomal aldolase activity Ariely Leite1, Antoniel Gomes2, Ana Sousa1, Marcos Fontes2, Sergio Schenkman1, Nilmar Moretti1 1) Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil; 2) Departamento de Biofísica e Farmacologia, Instituto de Biociências – UNESP – Universidade Estadual Paulista, Botucatu, SP, Brazil.

Post-translational modifications provide suitable mechanisms for cellular adaptation to environmental changes. Lysine acetylation is one of these modifications and occurs with the addition of an acetyl group to ε- amino chain of this residue, eliminating its positive charge. Recently, we found distinct acetylation profiles of procyclic and bloodstream forms of Trypanosoma brucei, the agent of African Trypanosomiasis. Interestingly, glycolytic enzymes were more acetylated in the procyclic, which develops in insects and uses oxidative phosphorylation to obtain energy, compared with the bloodstream form, whose main source of energy is glycolysis. Here, we investigated whether acetylation regulates the T. brucei fructose 1,6-bisphosphate aldolase. We found that aldolase activity was reduced in procyclic parasites cultured in the absence of glucose and partially recovered by in vitro deacetylation. Similarly, acetylation of protein extracts from procyclics cultivated in glucose-rich medium, caused a reduction in the aldolase activity. In addition, aldolase acetylation levels were higher in procyclics cultivated in the absence of glucose compared to those cultivated in the presence of glucose. To further confirm the role of acetylation, lysine residues near the catalytic site were substituted by glutamine in recombinant T. brucei aldolase. These replacements, especially K157, inhibited enzymatic activity, changed the electrostatic surface potential, decrease substrate binding and modify the catalytic pocket structure of the enzyme, as predicted by in silico analysis. Taken together, these data confirm the role of acetylation in regulating the activity of an enzyme from glycolytic pathway of T. brucei, expanding the factors responsible for regulating important pathways in this parasite.

200C Importance of Animal Models in Prevention, Control and elimination of malaria during Pregnancy: the Baboon Model of Placental Malaria Faith Ondtz1, Charles Owmandho2,3, Hastings Ozwara1 1) Institute of Primate Research, Nairobi, Kenya; 2) University of Nairobi, Nairobi, Kenya; 3) Kirinyaga University College, Kirinyaga, Kenya.

About 24 million pregnant women in sub-Saharan Africa are susceptible to malaria infection during pregnancy, a condition referred to as placental malaria. This susceptibility is greatest in their first and second pregnancies where malaria prevalence can be as high as 50%. Placental malaria contributes to poor pregnancy and birth outcomes which include low birth weight, intrauterine growth retardation, abortion, stillbirth, anemia, mortality, just to mention a few. Several factors such as the mother’s health status, inaccurate estimation of gestation, inadequate tissue for analysis, patient compliance, socio-economic conditions, and moral, ethical, and financial limitations make it difficult to perform comprehensive studies in human subjects. Therefore, reproducible animal models are required to overcome these challenges. The human-like structure of the baboon placenta and the cyto-adherent property of Plasmodium knowlesi have informed the choice for baboon-P. knowlesi model. Our findings explicitly demonstrate the infiltration and accumulation of infected P. knowlesi parasitized red blood cells and inflammatory cells within the intervillous space of the baboon placenta. This is a significant observation that describes placental malaria in P. falciparum-infected pregnant women. Further, we have demonstrated the role of IgG and cytokines in parasite clearance during pregnancy. Therefore, these findings validate and present this unique baboon-P. knowlesi model of placental malaria for further characterization and application in prevention, control, and elimination studies of malaria during pregnancy.

Keywords: Baboons, Model, Placental malaria, Plasmodium knowlesi.

201C A Plasmodium falciparum E3 ubiquitin ligase regulates transporter expression and drug responses Brojesh K Singh1, Jian wu1, Xiao He1, Keyla Tumas1, Yu-chih Peng1, Xin-zhan Su1 1) NIH/NIAID Rockville, Maryland.

Protein ubiquitylation is an important post-translational regulation, which has been shown to be necessary for life cycle progression and survival of Plasmodium falciparum. E3 ubiquitin ligases (E3s) are key players in protein ubiquitination and proteasomal degradation. Proteins playing a role in protein ubiquitination and ubiquitination, including E3 ligases, are potential drug targets. Here we generated transgenic P. falciparum parasite lines with specific E3 ubiquitin ligases tagged with pSli-HA-glmS sequence that enables us to knockdown (KD) the expression of E3 ubiquitin ubiquitin ligases. KD of an E3 ligase (E3-3) reduced ubiquitination of many parasite proteins. Changes in specific protein expression after E3-3 KD were identified using 2D gel electrophoresis and tandem mass spectrometry (MS-MS), and many of the proteins matched putative protein targets of artemisinin. E3-3 KD rendered parasites more sensitive to artemisinin derivatives and changed protein expression of P. falciparum multidrug resistant protein 1 (PfMDR1) and P. falciparum chloroquine resistance transporter (PfCRT). Therefore, the P. falciparum E3-3 ubiquitin ligase plays a role in parasite response to artemisinin derivatives and other antimalaria drugs by modulating the expression of key drug transporters and/or targets.

202C Mapping Cryptosporidium infection in vivo using 2-photon microscopy Bethan Wallbank1, Christoph Konradt1, 2, Christopher Hunter1, Boris Striepen1 1) University of Pennsylvania, Philadelphia, PA; 2) Purdue University, West Lafayette, IN.

From early diagnostic methods using light microscopy to cryo-EM of subcellular complexes, microscopic imaging has played a key role in advancing our understanding of parasitic diseases. The intestinal Apicomplexan, Cryptosporidium, is no exception to this. Electron microscopy has been essential to the
description of its ultrastructure and lifecycle progression. Recent molecular advances now allow us to endogenously express fluorescent markers and image molecular components of the parasite. However, with continued in vivo culture of Cryptosporidium largely not possible, the ability to visualize its full life cycle in vivo remains limited. Here we use 2-photon microscopy to visualize, for the first time, live Cryptosporidium in the mouse intestine. 2-photon microscopy allows for deep tissue imaging over extended periods of time. It negates the need for fixation and tissue clearing thus preserving natural tissue physiology and allowing for in situ imaging. We use a combination of intravitral and ex vivo 2-photon imaging methods to start to map the geography of infection throughout the intestine. This powerful tool will help us visualize the progression of infection, to map stage and species-specific tissue distribution, and to explore host-parasite interactions.

203C A mutagenesis screen to identify genes involved in T. gondii autophagy during chronic infection Fengrong Wang1, Geetha Kannan1, David Smith1, Tracey Schultz1, Vem Carruthers1 1) University of Michigan.

Toxoplasma gondii persists in humans by converting from active-replicating tachyzoites to slow-growing bradyzoites, which are encased in latent intracellular tissue cysts. Bradyzoites are impervious to existing treatments for toxoplasmosis, rendering chronically infected individuals at risk of progressive loss of vision or severe neural or pulmonary disease due to reactivated infection. Previously, our lab discovered that disrupting proteolysis within the parasite lysosome-like VAC/PLV led to accumulation of undigested autophagosomes and subsequent death of bradyzoites in culture and in infected mice. Although this suggested an important role for autophagy during chronic infection, the autophagic pathway is poorly understood in T. gondii and little is known about key regulators of the pathway. Also, several core components of autophagy found in model systems appear to be missing in T. gondii. To identify novel autophage players we performed a forward genetic chemical mutagenesis screen with type II Pru strain expressing tdTomato-ATG8 as a marker of autophagic flux and bradyzoite specific GFP. After in vitro conversion to bradyzoites we used FACS to capture the >95th percentile tdTomato-Atg8 of GFP positive bradyzoites. Four rounds of repeated differentiation and sorting resulted in an enriched population of mutant parasites that showed significantly higher tdTomato-ATG8 in bradyzoites than control parasites. Individual mutant clones were isolated and validated for abnormally elevated tdTomato-ATG8 fluorescence. We have performed whole genome sequencing on 45 mutants and obtained 8 independent clones. Each clone harbors 9 to 26 SNVs within coding regions, collectively encompassing 161 SNVs. For follow up studies we prioritized 16 SNVs that either appeared more than once in independent mutants or reside in genes that have literature precedent for being associated with autophagy in other systems. The properties of these genes and progress toward identifying phenotype driving SNVs therein will be presented as a steppingstone toward identifying key players in the autophagic pathway that impact chronic Toxoplasma infection.

204C Plasma Plasmodium falciparum Histidine-Rich-Protein-2 concentrations in children with various forms of clinical malaria in Kilifi, Kenya Sophie Uyoga1, Perpetual Wanjiku1, Jesse Rop1, Johnstone Makale1, Alexander Macharia1, Gideon Nyutu1, Charles Woodrow2, 3, Arjen Dondorp2, 3, Kathryn Maitland1, 4, Thomas Williams1, 4 1) KEMRI Wellcome Trust Research Programme; 2) Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University; 3) Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine; 4) Department of Infectious Diseases, Imperial College.

Background

Plasmodium falciparum histidine-rich protein 2 (PfHRP2) is produced by most strains of P. falciparum malaria parasites. Released from red blood cells at the time of schizont rupture, plasma concentrations of PfHRP2 reflect the total body parasite burden better than peripheral blood parasitaemia, because they also include the not circulating parasites sequestered in the microcirculation. Most, but not all, previous studies support a direct link between total parasite load and clinical severity of malaria. In the current study, we investigate the relationship between plasma PfHRP2 derived parasite load and falciparum malaria disease severity in children in Kilifi, Kenya.

Methods and findings

We estimated the burden of P. falciparum parasites in three groups of children with malaria infections of differing severity: (1) children admitted to our high dependency ward with WHO-defined severe malaria (n=1,544); (2) children admitted to the general paediatric ward with malaria but without features of severity (n=199) and; (3) children in the community with asymptomatic parasitaemia (n=28). Within hospital mortality was 11.7% in those with severe malaria and 0% in those with uncomplicated malaria. Peripheral parasite densities were measured by microscopy of venous blood films while total and sequestered parasite loads were estimated from plasma levels of PfHRP2 using previously established methods.

Results

Peripheral parasitaemias were highest in those with uncomplicated malaria (geometric mean 111,064; 95%CI 86,798-141,819 parasites/ml), being almost three times higher than those admitted with severe malaria (39,588; 34,990-44,791 parasites/ml) and more than 100 times higher than in those with asymptomatic malaria (1,092; 523-2,280 parasites/ml). However, geometric mean PfHRP2 values showed a clear step-wise increase across the groups, being 7 (4-12) mg/ml in children with asymptomatic malaria, 842 (655-1,084) mg/ml in children with uncomplicated malaria and 1,368 (1,243-1,505) mg/ml in children with severe malaria. More profoundly, the geometric mean sequestration index (SI; sequestered/circulating biomass) was approximately 10 times higher in children with severe malaria (SI 40.6; 35.7-46.0) than in those with either asymptomatic (SI 4.0; 1.8-8.6) or uncomplicated malaria (SI 4.9; 3.5-6.8)).

Conclusions

The clinical severity of malaria infections is strongly related to the total burden of P. falciparum parasites among children in Kilifi, Kenya. A quantitative test for plasma concentrations of PfHRP2 could be useful in identifying children at the greatest clinical risk.

205C RTP4 inhibits IFN-I response and enhances experimental cerebral malaria and neuropathology XIAO HE1, Xinzhuan Su1 1) NIAID/NIH.

Infection of malaria parasites triggers diverse immune responses leading to various symptoms and pathologies. Previously by using trans-species expression quantitative trait locus (Ts-eQTL) analysis, we identified clusters of host genes playing important roles in various pathways of immune responses previously. Here we investigate a gene encoding receptor transporter protein 4 (RTP4) known to promote cell surface expression of G protein-coupled receptors (GPCRs) and inhibit type I interferon (IFN-I) response. RTP4 binds to the TBK1 complex and interferes with TBK1 and IRF3 phosphorylation, leading to reduced IFN-I responses. Wild type (WT) and rtp4/-/- mice had similar parasitemia and mortality rates after infection with Plasmodium yoelii nigeriensis N67 parasites. In contrast, rtp4/-/- mice had significantly higher level of IFN-I response in microglia, lower parasitemia, fewer neurologic symptoms, and better survival than WT mice after infection with Plasmodiun berghei ANKA. Additionally, lower titers of west Nile virus (WNV) were observed in the brain tissue of rtp4/-/- than those of
206C Investigating the coding transcriptome of steady-state and reactivating exogenous stages of Eimeria tenella

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The major impacts of Eimeria spp. are to agricultural industries, where they cause the disease coccidiosis. This disease, and even the subclinical presence of parasites in farming operations, can severely impact the economic viability of such operations through reduced feed efficiency and slower animal growth, and, in severe cases of coccidiosis, significant mortalities. Research of Eimeria spp. has largely focused on development of new anticoccidials for broad application in animal farming operations. The unsustainability and the ongoing loss of efficacy of anticoccidial drugs indicate a need for alternative control strategies.

Development of new control strategies will require an improved understanding of the basic biology of Eimeria spp. The robust and environmentally resistant sporulated oocyst, the infectious stage disseminated into the environment, represents a gap in our understanding of the lifecycle of Eimeria spp. The hypoiodotic nature of sporozoites within the eimerian oocyst contributes to their ability to persist for extended periods of time. When the oocyst is ingested by the appropriate host, sporozoites within must quickly exit hypobiosis and prepare for the active penetration and establishment of infection within a host enterocyte. Both hypobiosis and rapid reactivation are critical aspects of the eimerian lifecycle. Indeed, these abilities appear to be features of all apicomplexan lifecycles. Research of the eimerian oocyst can therefore contribute not only to improving coccidiosis management strategies but could also be valuable in contributing to understandings of apicomplexan hypobiosis (and subsequent rapid reactivation).

We have deep sequenced the coding transcriptome of both steady-state and reactivating sporozoites of Eimeria tenella and present preliminary analyses herein. Heating hypoiodotic sporulated oocysts to the body temperature of their avian host to induce reactivation resulted in increased abundance of some transcripts that may be associated with sporozoite activities relating to excystation and host cell invasion. Other transcripts became less common suggesting that their products may be important in maintenance of the hypobiotic state of sporozoites within oocysts stored at ambient temperature. Trends and patterns in mRNA sequences and motifs of interest are reported, and candidates for genes supporting oocyst persistence and sporozoite reactivation are summarized.

207C 3D-cultures of Human Placental Trophoblasts Resemble the Maternal-Fetal Interface and Restrict Growth of Trypanosoma cruzi

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Trypanosoma cruzi (T. cruzi), the etiological agent of Chagas Disease (CD), is transmitted to humans by infected kissing bugs, blood transfusion, organ transplantation, and from mother-to-child. Vertical transmission of T. cruzi could perpetuate CD and has become a globalized public health challenge. With the implementation of successful blood donor screening strategies which significantly reduced the risk of transfusion transmitted infections, congenital transmission is now considered an important route of CD spread in non-endemic countries where no routine testing of pregnant women for the disease is implemented. Congenitally infected infants display mild or no symptoms of illness but may develop chronic CD with severe pathologies later in life if left untreated. The main cellular mechanisms that lead to fetal infection by T. cruzi, despite the presence of a placental barrier, remain unclear. Mother-to-child transmission most likely occurs when bloodstream trypomastigotes reach the placental intervillous space and interact with the large cellular surface provided by the syncytiotrophoblasts. These highly specialized cells not only function as a physical obstacle between mother and fetus, but also modulate immune responses against pathogen infections. In this study, we employed a three-dimensional (3D) cell culture system to replicate the human placenta environment in which trophoblast-derived JEG-3 cells are co-cultured with human brain microvascular endothelial cells (HBMEC) attached to beads, in a rotating wall vessel bioreactor. 3D culture of JEG-3/HBMEC spheroids promoted JEG-3 cell differentiation revealed by the formation of syncytia and production of b human chorionic gonadotropin and human placental lactogen. Further, 3D-grown JEG-3 cultures showed reduced susceptibility to T. cruzi infection compared to JEG-3 cells grown in tissue culture flasks (1% vs 20% of infected cells respectively). To identify cellular factors and pathways triggered during T. cruzi infection of 3D-JEG-3 cells, we performed gene expression and cytokine secretion profiling experiments. Preliminary data indicate changes in the expression of inflammatory chemokines and in genes involved in the innate immune response. Our trophoblast culture model overcomes the drawbacks of government regulations and ethical concerns related to the use of human tissues and could be useful to further study the mechanisms of parasite transmission and evaluate therapeutics to reduce congenital CD.

208C Plasmodium yoelii 17XNL infection induces macrophage dysfunction and blockage of erythrocyte maturation

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Malaria is caused by Plasmodium parasites which have symptoms ranging from non-symptomatic to deadly complications such as severe anaemia and severe malaria. Clearance of infected and uninfected erythrocytes and/or inhibition of erythropoiesis can lead to severe malaria anaemia. However, the exact molecular mechanisms of malaria induced anaemia are not completely understood. Using Plasmodium yoelii 17XNL parasites that invade reticulocytes and CS7BL/6 mice as a model, here we investigate the molecular mechanisms underlying malaria induced anaemia. Continuous decline in red blood cell (RBC) count, hematoctrit, and hemoglobin content was observed from day 4 to day 18 post infection (pi) with the 17XNL parasites. Increased numbers of erythroblastic islands (EBIs) with basophilic and polychromatic erythroblasts (TER119high, CD71high, FSChigh/low), elevated IL-12 levels, and reduced levels of IFN-gamma and TNF-alpha suggest activation of erythropoiesis in the spleen and bone marrow (BM). However, only a limited number of mature erythrocytes was produced from the spleen and BM, suggesting a blockage of erythrocyte maturation. Macrophages with increased expression of F4/80, EPOR, VCAM1, iNOS, and CD206 but decreased expression of CD163 and CD169 may have impaired function in supporting erythrocyte maturation. Additionally, removal of infected and possibly uninfected erythrocytes through phagocytosis and hemolysis likely contribute to anemia. This study reveals an important mechanism of malaria induced anaemia, which may be explored for treatments of severe malaria anaemia.

209C Structural Studies of the Harpoon-Like Invasion Organelle of Microsporidia

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Microsporidia are obligate intracellular, eukaryotic parasites capable of infecting a wide range of hosts from silkworms to immunocompromised humans. They do so through a fascinating organelle known as the polar tube which undergoes a rapid and tremendous conformational change from being tightly coiled inside the dormant spore to a long, linear tube out of the spore during infection. The filamentous polar tube is likely composed of repeating units of polar tube proteins (PTPs) whose structures remain a mystery, and which also don’t share sequence homology to any known protein. Furthermore, it
is not well understood how these proteins are arranged to form the tube. An understanding of the protein structures, as well as how they are arranged to potentially form repeating units and information on the ultrastructure of the tube will be key to understanding the invasion mechanism. We have optimized sample preparation for cryo-tomography and observed two different populations of the polar tube of microsporidia species Encephalitozoon hellem, with different visual characteristics. We are currently applying subtomogram averaging to improve the resolution of our model and optimizing recombinant polar tube proteins for X-ray crystallography. By taking advantage of the advances made in cryo-electron tomography and combining it with X-ray crystallography, we hope to understand how the architecture of the polar tube gives it robust material properties and facilitates infection.

210C *Plasmodium vivax* strains use alternative pathways for invasion

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*Plasmodium vivax* is the second most prevalent cause of malaria but remains understudied. During the blood stage of an infection, *P. vivax* parasites invade reticulocytes, the youngest red blood cells (RBCs). The invasion process is rapid and relies on multiple interactions between parasite invasion ligand proteins and specific host receptor proteins, termed invasion pathways. Disrupting these interactions is a promising approach for vaccine development. The related *P. falciparum* malaria parasite has numerous invasion pathways that are variably used and linked to immune evasion and pathogenesis. However, to date little is known about variant invasion pathway usage in *P. vivax*.

Currently only two *P. vivax* invasion pathways have been identified: the well-characterized interaction between *P. vivax* Duffy binding protein (PvDBP) and Duffy antigen receptor for chemokines (DARC); and the recently identified interaction between *P. vivax* reticulocyte binding protein 2b (PvRBP2b) and transferrin receptor (TFR1). To investigate invasion pathway usage, we developed a robust *P. vivax* invasion assay using cryopreserved Indian clinical *P. vivax* isolates. To reduce assay variability, we in vitro cultured isogenic reticulocytes from CD34+ bone marrow-derived hematopoietic stem cells.

To avoid complications with invasion ligand sequence variation, we used a host-targeted invasion inhibition approach. We used the cytokine melanoma stimulating growth activity (MGSA) to block the PvDBP/DARC interaction and we used an α-TFR1 monoclonal antibody OTK-9 to block the PvRBP2b/TFR1 interaction. We observed dose-dependent inhibition of each pathway, allowing us to estimate IC50 values for all 11 isolates that we tested. We observed 4.1-fold variation in inhibition of PvDBP/DARC and a greater 12.1-fold variation in inhibition of PvRBP2b/TFR1, demonstrating varied reliance on alternative invasion pathways by different *P. vivax* isolates, similar to what is seen in *P. falciparum*.

Variant invasion pathway usage may lead to reduced effectiveness of vaccine strategies targeting single antigens (PvDBP is a leading vaccine candidate). We tested for evidence of synergy by measuring invasion inhibition with different ratios of MGSA and OTK-9. Blocking both pathways together resulted in significantly increased invasion inhibition compared to each inhibitor alone, suggesting that a combinatorial vaccine strategy may be an attractive future approach.

The existence of an alternative invasion pathway system in *P. vivax* has implications for pathogenesis, immune evasion, cellular tropism and vaccine development.

211C Elucidating the role of receptor-type adenylyl cyclases in *Trypanosoma cruzi*

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*Trypanosoma cruzi*, the agent of Chagas disease, alternates between a vertebrate host and an insect vector throughout its life cycle. To survive drastic environmental changes this parasite differentiates into four main developmental stages. How does *T. cruzi* sense those changes, what type of stimulus triggers specific cellular responses that lead to cell differentiation, and are the main players in these signal transduction pathways, are some of the questions we pursue to answer. 3',5'-cyclic AMP (cAMP) is a universal second messenger that mediates cell differentiation in *T. cruzi*. However, this signaling pathway is poorly understood in trypanosomes. Adenylyl cyclases (ACs) catalyze the conversion of ATP to cAMP and have been reported to be calcium-stimulated in *T. cruzi*. ACs are structurally unique, with a single transmembrane domain and a catalytic domain located at the C-terminus of the protein, resembling mammalian calcium-sensitive guanylyl cyclases. These observations suggest that TcACs could link cAMP and calcium signaling pathways. These enzymes conform a multigenic family with 17 receptor-type members annotated in the *T. cruzi* Y strain genome. Using protein sequence alignment, we have classified them into five different groups, within which TcACs are highly conserved. We have chosen a representative member from each group to individually study their role (TcAC1-TcAC5). To establish their cellular localization, we overexpressed an HA-tagged version of each protein in different stages of *T. cruzi*. Immunofluorescence analysis shows a peculiar dual localization pattern of TcAC1 and 2 in the flagellar distal domain and in the contractile vacuole complex (CVC), while TcAC3 partially localizes to the ER, and TcAC4 and 5 localize to the CVC only. The adenylyl cyclase activity of TcAC1 and TcAC2 was confirmed by gene complementation in yeast. Furthermore, TcAC1 overexpressing parasites exhibit an increased metacyclogenesis of epimastigotes in vitro, a defect in host cell invasion by trypomastigotes and reduced intracellular replication of amastigotes, highlighting the importance of this protein throughout *T. cruzi* life cycle. We hypothesize that TcACs are responsible for cAMP synthesis in 2 different signaling domains: the contractile vacuole and the flagellar tip, where it senses nutrient deprivation, osmotic stress and cell contact to the vector’s hindgut epithelium, triggering cell adhesion and differentiation during *T. cruzi* metacyclogenesis.

212C Small ribosomal subunit rna (SSU/rRNA) sequence analysis of parasites isolates from patients diagnosed with visceral leishmaniasis with multiple relapses

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To perform sequence analyzes of the SSU/rRNA marker of other clinical isolates obtained from patients admitted at the same hospital diagnosed with VL, which
presented recurrent VL condition and non-response to treatment. The parasite isolation used bone marrow (BM) aspirates during hospitalizations and were maintained in cultures. The original patient’s polyclonal cultures were used to extracted genomic DNA of the promastigotes and to perform clonal isolation by plating. SSU/rRNA sequences were amplified using DNA from polyclonal and respective clonal samples by PCR. The amplicons were sequenced by the Sanger dideoxy method. The SSU/rRNA sequences (561pb) were analyzed by BLASTN and TriTrypdb resources. Phylogenetic analyzes were also performed including sequences of other trypanosomatids to build the trees. The results showed that the clinical isolates resembled other members of Leishmaniinae, such as the genus Crithidia and are identical to LHV60 strain. Continuous analysis are being carried out, including the sequencing of the complete genome and aiming at the future application of the real-time PCR technique to elucidate this possible co-infection and to develop accurate diagnostic methods.

213C Segmental changes in chromosomal allele frequency and loss of heterozygosity in Trypanosoma cruzi I. Lissa Cruz Saavedra1, Philipp Schwabi2, Gustavo Vallejo3, Marina Muñoz1, Luz Helena Patiño 1, Martin Lwelleny1, Juan David Ramírez González 1 1) Universidad el Rosario; 2) Universidad of Glasgow; 3) Universidad del Tolima.

*Trypanosoma cruzi*, the agent of Chagas disease shows tremendous genetic diversity and divided at least into six Discrete Typing Units (DTUs). A high intra DTU genetic variability has been observed in the Tci strains across the continent. Until now, the analysis of the Tci genetic architecture has been limited to the use of simple genetic markers and its genomic diversity and architecture deserves attention. Therefore, a genomic analysis of 19 clones produced from different *T. cruzi* I strains was performed by illumina platform. Phylogenetic analyzes demonstrated the presence of a unique genomic architecture of most of the Tci strains related to their geographical origin, on the other hand, the presence of a clade compatible with the Tcdom genotype described for strains from humans in Colombia and Venezuela was confirmed at a genomic scale. A total of three triploidy compatible segmental allele frequency patterns were found on chromosome 1. The presence of aneuploidy together with and loss of heterozygosity was observed in different chromosomes. Our result suggested high genomic plasticity and the presence of random events of recombination during complex biology in the *T. cruzi* lifecycle.

214C Adipose Tissue Resident *Trypanosoma brucei*: a Population of Slow Growers Sandra Trindade1, Mariana Sequeira1, Mariana De Niz1, Mario Dejung2, Fabio Bento1, Daniel Pinto-Neves1, Falk Buter2, Frédéric Bringaud3, Erda Gijn4, Luis M. Figueiredo1 1) Instituto de Medicina Molecular João Lobo Antunes (iMM), Lisboa, Portugal; 2) Instituto de Molecular Biology (IMB), Mainz, Germany; 3) University of Bordeaux, Bordeaux, France; 4) Instituto Gulbenkian de Ciência (IGC), Oeiras, Portugal.

During a mouse infection, *Trypanosoma brucei* occupies the adipose tissue by a particular adaptation process. Yet, knowledge on the role of this population on the parasite load dynamics is still scarce. By integrating in a single mathematical model the total number of parasites and the proportion of stumpy forms on blood and adipose tissue overtime, we established parameter differences between the two compartments. The best-fit model estimated that the proliferation rate of replicative forms in the adipose tissue is 50% lower than their blood counterparts. The comparison of the cell cycle profiles of the two parasite populations, showed that adipose tissue harbors a higher percentage of parasites in G1 (56% vs 52.5%), suggestive of a lower proliferation rate. Intravital microscopy of mice infected with CellTraceTM Violet (CTV) labeled parasites corroborated that Adipose Tissue Forms (ATFs) divide on average 50% slower than Bloodstream Forms (BSFs). To better define the differences between blood and adipose tissue populations we compared the proteomes of isolated parasites. Among the 2800 identified protein groups, 6% proved to be differentially expressed. In ATFs, most downregulated proteins are involved in protein synthesis and ribosome biogenesis, suggesting that ATFs may have a lower protein synthesis rate. FACS analysis of parasites labeled with L-Homopropargylglycine (HPG) confirmed that protein synthesis is 24% lower in ATFs than BSFs. Together, these findings identify the adipose tissue as a niche for slow growing parasites with reduced protein synthesis. This behavior could be important to reduce pathogenesis and increase the chances of parasite survival and transmission. Importantly, these findings may have strong implications for drug treatment because drugs may not be equally effective at eliminating parasites in the blood and parasites colonizing other organs.

215C Identifying Amphibian Pathogens in the Field Using Quantitative PCR Ana Cvetkovic1, Lauren Bode1 1) North Central College.

Water molds are widespread amphibian pathogens. We developed a quantitative PCR (qPCR) assay that detects and quantifies the DNA of water molds from field samples. We tested the efficacy of two types of qPCR probes that were designed to bind to a particular sequence of water mold DNA Minor Groove Binding (MGB) probes and Locked Nucleic Acid (LNA) probes. The two probes use different mechanisms to bind to double-stranded DNA present in a qPCR reaction. In our study, both set of probes successfully amplified targeted DNA, but only in high concentrations of DNA. Due to its higher specificity to target DNA sequences, LNA probes are a promising tool for identifying microbes from field samples.

216C Gene disruption of flagellar and cytoskeleton components of Trypanosoma cruzi. José Sáenz1, Normanda Souza2, Beatrix Borges3, Lisandro Pacheco4, Rodrigo Monte-Neto5, Stenio Fragoso6, Richard Wheeler 7, Nilmar Moretti8, Lia Soares9, Wanderson Darocha10 1) UFPR; 2) UNIFESP; 3) Instituto Carlos Chagas (ICC); 4) Universidade Simón Bolivar; 5) Instituto Carlos Chagas (ICC); 6) Instituto Carlos Chagas (ICC); 7) University of Oxford-Medical Sciences Division; 8) UNIFESP; 9) Instituto Carlos Chagas (ICC); 10) UFPR.

Parasite cytoskeleton and flagellum are key structure for its survival and virulence. Recent reports have shown that parasite flagellum is not only required for parasite swimming, but it plays role on interaction with host structures/organelles, social motility, and cytokinesis. To identify conserved flagellar components among Trypanosomatids shared by *Trypanosoma brucei* we performed in silico analysis using TriTrypDB data. Flagellar proteomic data from *Trypanosoma brucei* were used to identify orthologous genes in *T. cruzi*. For these genes, we analyzed RNAseq data to access its mRNA expression profile through amastigote to trypomastigote transition during infection. The genes that were conserved and the RNAseq data was compatible with flagellum size were selected for functional characterization, since they could be potential candidates for drug development or to attenuate parasite virulence. Aiming to functionally dissect the essential genes encoding flagellar proteins, we are developing tools such as gene editing, conditional knock out and protein tagging. CRISPR/Cas9 was applied for functional characterization, since they could be potential candidates for drug development or to attenuate parasite virulence. Aiming to functionally dissect the essential genes encoding flagellar proteins, we are developing tools such as gene editing, conditional knock out and protein tagging. CRISPR/Cas9 was applied to edit two conserved proteins Kharon1 (cytoskeleton), and Trypanin (flagellar protein), which were previously characterized in two related species, *T. brucei* and *Leishmania mexicana*. Edited parasites show interesting phenotypes, Kharon1 disrupted population show morphology alterations, and reduced growth, while Trypanin depleted has decreased motility. To confirm their function, we are developing strategies to tag these proteins using easy-to-clone plasmid and conditional complementation using CRE recombinase system. We plan to use these strategies to dissect *T. cruzi* flagellum, which is poorly studied its function in biological processes of these neglected parasite.

217C *Cyclospora cayetanensis* whole genome sequencing: Improving the quality and diversity of available genomes Christine Vonto1,2, John Bart1, Rebecca Guy2 1) Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada; 2) Division of Enteric Diseases, National Microbiology Laboratory at Guelph, Public Health Agency of Canada, Guelph, Ontario, Canada.

*Cyclospora cayetanensis* is an emerging food-borne, human pathogen causing cyclosporiasis, an enteric disease, in individuals worldwide. Despite a growing trend of national outbreaks in North America, there are no routine, efficient laboratory methods for strain-level characterization to assist in outbreak investigations. Genomic material must be analyzed directly from stool specimens to properly subtype this parasite because *C. cayetanensis* cannot be propagated in laboratory settings and isolated as a pure culture. Fortunately, recent advances in Next Generation Sequencing technologies have provided the opportunity to obtain high quality genetic information from this parasite. There are 37 short-read assemblies available publicly from seven countries but none of these assemblies are complete. To improve both quality and diversity of the available genome assemblies for comparative genome analyses, we attempted to generate
the first whole genome sequence of Canadian C. cayetanensis isolates using both long-read and short-read sequencing technologies. We purified the oocysts from human stool using a series of gradients to remove contaminant organisms, and incorporated a whole genome amplification procedure to obtain an adequate quantity of DNA for successful sequencing. Our preliminary results show that including two sucrose-based gradients, a cesium chloride gradient, and a bleach wash purified the oocysts sufficiently so that less than 10% of sequencing reads were from contaminant species. However, we were unsuccessful at amplifying DNA in sufficient quantities across the entire genome for long-read sequencing using a generic, random hexamer based whole genome amplification method. Therefore, we suggest that a more targeted approach is required to sufficiently amplify all regions of the genome. Overall, the addition of Canadian Cylcospora cayetanensis genomes to public databases will allow for a comparative genomic investigation between other countries’ isolates to develop further subtyping methods to provide valuable tools for public health agencies in outbreak investigations.

218C Discovery of a novel and essential member of the Plasmodium falciparum basal complex. Alex Moreano1, Rachel Rudlaff1, Jeffery Dvorin1 1) Harvard Medical School.

In the asexual stage of the Plasmodium falciparum life cycle, where the parasite replicates in red blood cells and causes malaria’s characteristic cyclical fevers, a ring-like structure known as the basal complex is required for replication and serve to separate budding daughter cells. We have discovered a novel member of the basal complex, PF3D7_1018200/PfPPP8, which colocalizes with known basal complex proteins PfMORN1 and PICINCH and is essential for parasite growth and division. Examining epitope-tagged PfPPP8 and the phenotype of PfPPP8-knockdown parasites has revealed unique features about the essential protein that distinguish it from other basal complex proteins. PfPPP8 protein is degraded before final constriction of the basal complex at the very basal end of the parasite, and unlike PICINCH, PfPPP8’s knockdown completely abrogates formation of the basal complex. Thus, we hypothesize that PfPPP8 plays a vital role specifically in the assembly and organization of the basal complex during the earlier stages of asexual division.

In silico analysis identified a putative serine-threonine phosphatase and a Ca2+ binding EF-hand domain as regions in the protein. To determine whether these conserved domains are essential for protein function, we will utilize genetic complementation in parasites with inducible knockdown in endogenous PfPPP8. We will examine PfPPP8’s enzymatic activity in vitro with recombinant forms of the protein. Because PfPPP8 is essential for parasite growth and replication during the pathogenic stage and because it is conserved only among parasitic apicomplexans, further study could prove it a useful new target for developing antimalarials in the face of rising resistance to extant drugs.

219C Discovery of novel proteins within the Plasmodium falciparum inner membrane complex. Ana Karla Cepeda Diaz1,2, Rachel Rudlaff1,2, Jeffrey Dvorin2,3 1) Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts; 2) Division of Infectious Diseases, Boston Children’s Hospital, Boston, Massachusetts; 3) Department of Pediatrics, Harvard Medical School, Boston, Massachusetts.

During the clinically important asexual blood stage of P. falciparum replication, the parasite develops via schizogony, undergoing repeated nuclear divisions to produce a multi-nucleated cell. This is followed by segmentation, a specialized cytokinesis, during which individual nuclei and associated organelles are partitioned to produce daughter parasites. The inner membrane complex (IMC), the associated basal complex, and the interaction of these structures with parasite nuclei are critical for segmentation. The IMC is a unique membranous structure with associated proteins inside the parasite that dictates its shape and rigidity. Attempts to identify and characterize the proteins that make up the P. falciparum IMC remain limited. We have evaluated the functional role of PF3D7_0525800/PfIMC1g, an alveolin-repeat containing protein, during asexual parasite development. We demonstrate that PfIMC1g is essential and that its knockdown leads to defects in both segmentation and parasite development. We performed a co-immunoprecipitation (co-IP) using PfIMC1g as bait followed by unbiased mass spectrometry to identify IMC1g binding partners. Among our top hits, we identified a putative RNA-binding protein, PF3D7_1310700, which was confirmed to localize to the IMC by confocal microscopy. A reciprocal IP using this putative RNA-binding protein as bait followed by an immunoblot confirmed that IMC1g co-immunoprecipitates with it. We conclude that PfIMC1g binds directly or indirectly to a putative RNA-binding protein localized at the IMC. Ongoing experiments will evaluate the functional role of this novel IMC protein.

220C Investigating Variant Surface Glycoprotein dynamics under inhibition of fatty acid synthesis in Trypanosoma brucei. Nava Poudyal1, Kimberly Paul1 1) Clemson University.

Trypanosoma brucei is a hemoflagellate that causes African Sleeping Sickness in humans and the wasting disease nagana in cattle, both considered neglected tropical diseases. The T. brucei surface is coated with 107 identical copies of single Variant Surface Glycoprotein (VSG), one of ~2500 VSGs encoded in the genome. VSG helps T. brucei evade host immune response by two mechanisms: (1) antigenic variation, in which the parasite periodically changes its VSG coat; and (2) endocytosis, in which VSGs bind to serum antibodies and complement are internalized, and VSG recycled while serum proteins are transported to lysosome for degradation. For both strategies, the VSG glycosylphosphatidylinositol (GPI) membrane anchor and its two myristate fatty acids are thought to play a key role in VSG mobility and trafficking. The role of fatty acid synthesis (FAS) in VSG function is unknown but is likely needed to maintain VSG GPI anchor myristoylation. To reduce FAS, we use RNAi mediated depletion of Acetyl-CoA Carboxylase (ACC), the first enzyme in the FAS pathway. We found that ACC RNAi resulted in a significant 30-40% reduction in both receptor-mediated and fluid phase endocytosis. To determine the effect of ACC RNAi on VSG dynamics, we first immunoprecipitates with it. We conclude that PfIMC1g binds directly or indirectly to a putative RNA-binding protein localized at the IMC. Ongoing experiments will evaluate the functional role of this novel IMC protein.

221C Plasmodium vivax infection destabilizes the host reticulocyte. Martha Clark1, Usheer Kanjee1, Gabriel Rangel2, Laura Chery3, Anjali Mascarenhas4, Edwin Gomez4, Pradipsinh Rathod3, Carlo Brugnara5, Marcelo Ferreira6, Manoj Duraisingh1 1) Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, 655 Huntington Ave, Boston, MA 02115 USA; 2) Department of Chemistry, University of Washington, 3781 Okanogan Ln, Seattle, WA 98195 USA; 3) Department of Biochemistry and Molecular Biology, Pennsylvania State University, W126 Millennium Science Complex University Park, PA 16802 USA; 4) School of Public Health, 655 Huntington Ave, Boston, MA 02115 USA; 5) Department of Parasitology, Instituto de Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 2415 - Butantã, São Paulo - SP, 05058-900, Brazil.

The structural integrity of the host red blood cell (RBC) is maintained for successful propagation of Plasmodium spp. parasite blood stage infections. Osmotic stability is an indicator of RBC structural integrity and is used clinically to diagnose RBC disorders associated with hemolytic anemia such as hereditary spherocytosis. Plasmodium vivax infection imposes a strict tropism for the youngest of red blood cells (RBCs), reticulocytes. The inherent osmotic stability of reticulocytes and the subsequent impact of P. vivax on reticulocyte osmotic stability are unknown. To assess the osmotic stability of these inherently heterogeneous RBC populations, we developed a single cell flow cytometry-based osmotic stability assay. We then examined the osmotic stability of reticulocytes and RBC precursors from bone marrow aspirates and in vitro RBC cultures. We found that reticulocytes and RBC precursor osmotic stability decreased during erythropoiesis and reticulocyte maturation, with P. vivax preferred young reticulocytes being the most stable of the enucleated populations examined. To determine the impact of P. vivax infection on reticulocyte stability, we examined the osmotic stability of short-term cultured clinical Indian and Brazilian P. vivax isolates. We found that as P. vivax progressed through the IDC, the osmotic stability of infected-reticulocytes decreased to levels

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similar to those observed for RBC disorders associated with hemolytic anemia. Furthermore, when we compared the osmotic instability of \( P. \) vivax infected-reticulocytes and \( P. \) falciparum infected-normocytes, we found that \( P. \) vivax infected-reticulocytes were significantly less stable than \( P. \) falciparum infected-normocytes. Finally, we investigated the contribution of \( P. \) vivax new permeability pathways (NPPs) to the osmotic instability of infected-reticulocytes. We found that susceptibility of \( P. \) vivax infected reticulocytes to lysis by NPP antagonists D-sorbitol and L-alanine corresponded with decreasing osmotic stability of \( P. \) vivax infected reticulocytes, suggesting that \( P. \) vivax NPPs contribute to the decreased osmotic stability of infected-reticulocytes. Together these results reveal a key vulnerability of \( P. \) vivax that may be a mechanism by which RBC polymorphisms like hereditary spherocytosis and G6PD deficiency provide protection from malaria and additionally could be manipulated to yield both in vitro culture and novel therapeutics.

222C Post-pairing transcriptome analysis of \( Schistosoma mansoni \) identifies a key mediator in the male parasites for female reproduction development stimulation \( Rui Chen \), James Collins 1) UT Southwestern Medical Center.

Schistosomes are blood sucking parasitic flatworms living in mammalian hosts and causing serious chronic diseases solely due to their enormous egg output. Of our particular interest on their basic reproductive biology, which is directly pathology related, is an observation that female worms rely on continuous pairing with male worms to develop and maintain a mature reproductive system and to sustain egg production. This process is not sperm-transfer dependent and completely reversibly depending on the presence of physical contact from male parasites. Multiple transcriptome comparisons between pre-and post-pairing female parasites from our lab revealed that the majority of the molecular changes happening to female worms before signs of anatomical sexual maturation were in somatic tissues. On the male side, only a limited list of regulated genes upon pairing was seen. The most up-regulated genes in male parasites after pairing, SmNRPS; was predicted to encode a Nonribosomal Peptide Synthetase (NRPS) that shares 36% similarity to the Drosophila NRPS Ebony. A SmNRPS RNAi male worm lost its ability to induce female worm to her best sexual maturity. In situ hybridization showed that SmNRPS was expressed only in the ventral surface neuronal cells of paired male parasites. This surface is in direct physical contact with female parasites when a pairing position (in copula) is presented. From the above data, we believe SmNRPS is a critical factor between the female/male interplay interface that mediates the stimulation for female reproduction development. The ongoing project is to identify the biochemistry nature of SmNRPS as a potential nonribosomal peptide synthetase. We wish to characterize its amine substrates and nonribosomal peptide product(s) following the successful biochemical strategies applied to Drosophila Ebony. Metabolomics approaches comparing monoaomic landscapes after pairing and following SmNRPS ablation will be conducted too. In the end, we hope to discover the stimulus molecule(s) that is sufficient to induce female schistosome maturation.

223C Leucine Carboxyl Methyltransferase (LCMT) from \( Leishmania infantum \) and its interactome. \( Arijit Bhattacharya \), Akash Mitra 1) Adams University.

Leucine carboxyl methyl transferases (LCMT) mediate transfer of methyl group from S-adenosyl methionine (SAM) to carboxyl group of C-terminal leucine (L) in proteins. The \( Leishmania infantum \) orthologue of LCMT was identified by genome wide gain of function screen for resistance against sinefungin (SNF), an antibiotic and structural analogue of SAM. LCMT from \( L. \) infantum (LилLCMT) is phylogenetically distant from eukaryotic homologues with conserved catalytic motifs. WT cells epoxially overexpressing lcmt and LCMT/-/ cells showed resistance against SNF in a growth stage dependent manner. Molecular docking analysis with a valid homology model for LCILMT revealed considerable interaction with known methyl transferase inhibitors. With more than 10% proteins possessing C-terminal L residue in \( L. \) infantum proteome, LCMT might play crucial role in regulating number of processes. To identify candidate interactome, immunoprecipitation coupled to LC-MS/MS analysis was implemented, which identified 46 proteins with C-terminal L residue. GO-enrichment analysis revealed enrichment of "translation factors". In silico protein-protein docking analysis showed substantial interaction of several translation associated proteins with LCMT. The results warrant further exploration of LCMT mediated regulation of ribosomal activity in the parasite.


The dynamics of pathogens between domestic and wild fauna remain poorly understood. Numerous conservation problems emerge on that border. Concerning this matter, genomes are used to design of new molecular tools for the sensitive detection of helminths that cause risk diseases from domestic to wild carnivores. \( Dicrotophyema renale \) is a parasitic nematode that infects the kidney of mammals (mainly Order Carnivora). It can grow and exceed the meter, destroying the kidney. Therefore, its infection is strongly debilitating and even fatal. Numerous cases in domestic dogs were observed on the Argentine coast wild carnivores.

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Potentially zoonotic nodular worms infecting free-ranging non-human primates in Kenyan urban centres: potential reservoirs for human infections \( Peris Mbuyi \), Edwin Murungi2, Vincent Owinio1, Mercy Akinji3, Gillian Eastwood4, Richard Nyamota1, Isaac Lekolool5, Maamun Jeneby3 1) Adamas University. Arijit Mitra1 1) UT Southwestern Medical Center.

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From genome Project (developed by our laboratory), a mitochondrial genome of \( D. \) renale was assembled and three molecular markers of different sizes from cytochrome oxidase I (COX1) gene were designed. Forty-six adults of \( Dicrotophyema renale \) were collected from interventions in veterinary clinics and from necropsies of wild fauna in different locations in northeastern Argentina and southern Brazil. DNA was extracted from each sample and those markers were amplified by PCR. The amplified products were typed by SNPs analysis and phylogenetically analyzed with sequences available in the GenBank, which came from Japan, Peru, Canada and Iran. Ten local genetic variants of South America were observed, without geographic differentiation among the localities of Argentina, Peru and Brazil, but a great separation with regard to the sequences of other regions of the world. Nor was any structuring by host species observed. This could be similar to those observed for RBC disorders associated with hemolytic anemia. Furthermore, when we compared the osmotic instability of \( P. \) vivax infected-reticulocytes and \( P. \) falciparum infected-normocytes, we found that \( P. \) vivax infected-reticulocytes were significantly less stable than \( P. \) falciparum infected-normocytes. Finally, we investigated the contribution of \( P. \) vivax new permeability pathways (NPPs) to the osmotic instability of infected-reticulocytes. We found that susceptibility of \( P. \) vivax infected reticulocytes to lysis by NPP antagonists D-sorbitol and L-alanine corresponded with decreasing osmotic stability of \( P. \) vivax infected reticulocytes, suggesting that \( P. \) vivax NPPs contribute to the decreased osmotic stability of infected-reticulocytes. Together these results reveal a key vulnerability of \( P. \) vivax that may be a mechanism by which RBC polymorphisms like hereditary spherocytosis and G6PD deficiency provide protection from malaria and additionally could be manipulated to yield both in vitro culture and novel therapeutics.

Natural infections with helminths occur in non-human primates (NHPs) and have the potential to cross primate-species boundaries and infect humans causing diseases of public health concern. Of concern are soil-transmitted helminths (STHs) whose partial development outside of their hosts allow persistence of infective stages in the environment and enable transmission between closely related host species to occur within immediate temporal overlap. Consequently, sharing of habitats and watering points by humans and NHPs is potentially important in the transmission of zoonotic STHs. Relevant to this context
is Oesophagostomum species considered endemic to west African countries. Its transmission potential between human and NHPs under natural conditions has been a source of considerable debate. In Ghana, the identification of genetic differences among Oesophagostomum nematodes infecting humans and population of baboons in the same region suggested that the parasite is not commonly cross transmitted. However, a novel Oesophagostomum clade infecting human and five sympatric species of NHPs was recently described in Uganda. While great apes harbour O. stephanostomum, two cases have been reported in humans. This underscores the importance of local scale research for zoonosis risk and epidemiology of this oesophagostomosis and the role of NHPs as potential reservoirs of the infection in Eastern Africa. Additionally, clinical consequences of oesophagostomosis necessitates elaborate investigation of the etiological agents. Previous surveys for helminths in free-ranging NHPs have focused on Kenya’s wildlife reserves and rural forest habitats. This bias has resulted in little knowledge on zoonoses within urban habitats that sustain NHPs, which could be incidental hosts or reservoirs of emerging helminths.

This study investigated the species diversity of nodular worms in free-ranging NHPs in urban and peri-urban centres in Kenya. Polymerase chain reaction (PCR) coupled with high-resolution melting (PCR-HRM) analysis and sequencing were used to identify nodule worms. Phylogenetic analysis showed that O. stephanostomum had a close evolutionary relatedness to human isolates suggesting the zoonotic potential of this parasite. Moreover, we also report the first natural co-infection of O. bifurcum and O. stephanostomum in free-ranging vervets.

Thus, preventative integrated health strategies to counter urban oesophagostomiasis need to be instituted.

226C Nanobodies as biological tools to characterize the 6-cysteine proteins of Plasmodium falciparum and to develop interventions to inhibit parasite infection Melanie H Dietrich1,2, Li Jin Chan1,2, Amy Adair1, Sravya Keremane1, Alvin W. Lo1, Yichun Cao1, Wai-Hong Tham1,2 1) Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; 2) Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia.

Surface-associated proteins play critical roles in the Plasmodium parasite life cycle and are major targets for vaccine development. The 6-cysteine (6-cys) protein family has 14 identified members, which are expressed in stage-specific manner throughout the parasite life cycle. This protein family is conserved across Plasmodium species and is characterized by domains of ~120 amino acids that contain positionally conserved cysteines that form disulfide bonds. Although some 6-cys members have been implicated to play a role in sexual stages, mosquito transmission, evading the host immune response and as invasion ligands, the precise function of many family members is still unknown and structural information is only available for four 6-cys proteins. We are interested in further dissecting the roles of the 6-cys family during the multiple stages of the parasite life cycle. To do so, we have expressed and purified 6-cys protein family members for structural studies that will provide high-resolution crystal structures. To assist with the functional characterization of this important protein family, we are in the process of isolating single domain antibodies from immunized alpacas to each member. These single domain antibodies can be used to examine the cellular localization of 6-cys proteins, to identify interacting partners and provide opportunities to identify neutralizing epitopes against parasite blood stage invasion and transmission between human and mosquito. These studies will shed light on the diverse functions of the 6-cys family in the Plasmodium life cycle and provide opportunities to develop interventions that may inhibit parasitic infection.

227C Small molecule targeting of an essential Plasmodium CDK enzyme Aditya Paul1, Fleur Ferguson2, Tinghu Zhang2, Arianna Bonilla1, Jinhua Wang2, Nathanael Gray2, Manoj Duraisingh1 1) Harvard T.H. Chan School of Public Health, Boston, MA; 2) Dana-Farber Cancer Institute, Boston, MA.

Cyclin-dependent kinases (CDK) regulate pathways for cell cycle progression and proliferation in eukaryotic organisms. In Plasmodium spp., genetic studies show that parasite CDK homologs are essential for asexual proliferation. Here, we utilized a structurally diverse set of compounds predicted to target a broad spectrum of CDK enzymes to probe erythrocytic-stage cellular processes regulated by Plasmodium CDKs and small molecule-susceptibility. We find that Plasmodium falciparum parasites are sensitive to these inhibitors with IC50 values ranging between ~0.1 and 10 µM. We assessed the stage-specificity of action of inhibitors using morphological criteria, observing blockade of intraerythrocytic development either at an early stage before substantial cellular growth, or during DNA replication at the schizont-stage. We used transgenic parasites for conditional knockdown of the essential S-phase parasite enzyme Pfcd2c-related kinase (CRK4) to assess targeting of PfCRK4-regulated schizogony by CDK-targeting chemical inhibitors. We observed chemical-genetic functional interactions between PfCRK4 and subsets of molecules. Compounds exhibiting synergy with PfCRK4 arrest parasites either immediately before or during DNA synthesis. Flavopiridol/alogocidin, a flavonoid with broad-spectrum human CDK activity, blocked parasites early in schizogony, similar to the phenotype elicited by reverse-genetic knockdown of PfCRK4. A derivative of the CDK inhibitor AT7519 similarly phenocopies PfCRK4-knockdown. In contrast, growth-inhibitory compounds exhibited antagonism with PfCRK4. Our analysis suggests that parasite homologs of CDK enzymes can be targeted, and further may indicate molecular leads for specifically targeting PfCRK4.

228C The chaperone PbHscB plays a crucial role in Plasmodium berghei liver stage development Raphael Beyerler1, Rebecca Stanway1, Magali Roques1, 2, Volker Heusler1 1) Institute of Cell Biology, University of Bern, Switzerland; 2) Uniscientia Stiftung, Vaduz, Liechtenstein.

The liver stage of Plasmodium spp. represents a bottleneck in the life cycle of the parasite and is therefore an important target for drug and vaccine development. In order to identify novel targets, a high-throughput knockout screen was performed that identified 185 parasite genes that are required for normal liver stage development (Stanway et al. 2019). Several molecular chaperones were identified including the mitochondrial iron-sulphur assembly co-chaperone PbHscB. According to the results of the screen, individual PbHscB knockout parasite clones exhibited a marked delay in liver stage development both in vitro and in vivo. Despite the strong liver stage phenotype of PbHscB-KO parasites, some parasites can still complete liver stage development, indicated by a delayed expression of merozoite surface protein 1 (MSP1) in vitro. When infecting mice with PbHscB-KO sporozoites, a delay in prepatency of 2-4 days was observed, confirming the in vitro obtained results. Interestingly, endogenous GFP-tagging revealed localization of PbHscB in the parasite mitochondrion, suggesting an important function in energy supply or in metabolic processes. We therefore hypothesize that therapeutic approaches that specifically target the host, rather than the pathogen itself present a valid therapeutic option to reduce parasite burden and ultimately, treat visceral leishmaniasis.

We aimed to use L. donovani in both in vivo and in vitro systems to explore the role of different cell death pathways in visceral leishmaniasis.
Using gene-targeted mice as well as therapeutic compounds targeting the host cell apoptotic and pyroptotic machinery, we determined parasitic burden, cell death and protein expression through microscopy, live cell imaging, flow cytometry, immunohistochemistry and immunoblotting upon infection with *L. donovani*.

Our results suggest there is no role for pyroptosis as Caspase-1/11/12/- and Gasdermin-D/- mice also showed no difference in parasite burden compared to C57Bl/6 controls. Additionally, pharmacological induction of intrinsic apoptosis using BCL-2, MCL-1, BCL-XL inhibitors also do not affect parasite burdens in vitro.

However, extrinsic apoptosis appears to play a central role. Inducing extrinsic apoptosis of infected murine bone marrow derived macrophages with IAP inhibitors resulting in parasite death. Mice infected with *L. donovani* and treated with IAP inhibitors have a reduced leishmaniasis symptoms, and splenic parasite reservoir due to apoptosis of the parasite reservoir cells, macrophages. Furthermore, combining IAP inhibitors with the standard therapy for visceral leishmaniasis, Amphotericin-B, enables reduction in the concentration of both therapies, and still allows significant reduction in splenic parasite burden.

This indicates targeting host apoptotic pathways may be a valid therapeutic option for visceral leishmaniasis.

230C Hi-C reveals dynamic interactions between the active VSG gene and a potential, unconventional enhancer *Vanessa Luzak*1, Joana Faria2, Laura S.M. Müller1, Benedikt G. Brink1, Sebastian Hutchinson3, Lucy Glover3, David Horn2, T. Nicolai Siegel1 1) Ludwig-Maximilians-Universität München, Munich, Germany; 2) University of Dundee, Dundee, United Kingdom; 3) Institut Pasteur, Paris, France.

Specific cis-regulatory interactions of enhancer sequences with their target promoters play an important role for gene expression regulation in metazoa. An enhancer serves as a recruiting platform for transcription factors, cofactors and active polymerases and the transient interaction with a promoter selectively initiates transcription of the respective gene.

In contrast, transcription initiation occurs mostly unregulated in trypanosomes. Yet, trypanosomes rely on highly selective VSG gene expression to undergo antigenic variation and survive in the mammalian host. Transcription initiation by Pol I is common for all VSG expression sites, but elongation and transcript processing occurs exclusively at one expression site, resulting in monogenic VSG expression. Recently, we found that an intact genome organization is required for selective VSG expression. Using chromosome conformation capture (Hi-C), we have now identified specific inter-chromosomal interactions that enable exclusive transcript processing at only one expression site.

Hi-C analysis revealed that the active VSG gene was located in a transcriptionally active nuclear compartment, where it interacted with the Spliced Leader (SL) array, a genomic locus coding for the essential trans-splicing substrate SL-RNA. Inactive VSG genes resided in a transcriptionally silent nuclear compartment and did not show elevated interaction levels with the splicing locus. Further, we analyzed genome-wide interaction patterns after a switch in VSG expression and after simultaneous upregulation of multiple VSG genes. We found in both cases that activated VSG genes transitioned from the transcriptionally silent to the active compartment, where they interacted with the SL-array, indicating that the interaction with the splicing locus is dynamic and plays an important role for VSG gene activity.

Besides VSG genes, procyclin genes are highly transcribed by Pol I and indeed, we also found procyclin genes to interact with the SL-array upon activation in procyclic cells. Surprisingly, also Pol II transcribed housekeeping genes interacted with the SL-array, supporting a universal mechanism that evolved in trypanosomes to establish high levels of Pol I and Pol II transcribed mRNAs. Our data suggest that the SL-array serves as an unconventional “post-transcriptional” enhancer, selectively enhancing transcript levels of genes in close proximity by facilitating RNA processing rather than transcription initiation.

231C Allele-specific genome assembly of the Trypanosoma brucei Lister 427 isolate *Raúl Oscar Cosentino*1, Benedikt Brink1, Nicolai Siegel1 1) Department of Veterinary Sciences, Ludwig-Maximilians-Universität München, Munich, Germany.

Using PacBio long reads, we recently generated a genome assembly of the *T. brucei* Lister 427 isolate. In this assembly, the haploid-like subtelomeric regions, harbouring the repertoire of variant antigens, were assembled in an allele-specific manner. Nevertheless, the ‘cores’ of the chromosomes, encoding most of the active transcriptome, were assembled in a collapsed-manner, disregarding the information of allelic variants and the association between them.

In this project, we decided to analyse the variant distribution in the Lister 427 ‘core’ genome and to deliver a fully phased genome of the most widely used isolate in the *T. brucei* research field.

First, we mapped short-read error-corrected PacBio reads to our genome assembly to identify the heterozygote variant positions. Then, to link the variants, we used HapCut2, a tool capable of integrating information from different sequencing technologies, to construct haplotype blocks, feeding it with raw PacBio and chromosome interaction (Hi-C) data.

With this approach, we identified ~96 thousand allelic variants across the genome ‘core’ (average of ~4 variants/Kb), and were able to confidently assign ~97% of them to a specific chromosome allele. We found that the variant density along the chromosomes was very uneven, with several long regions of loss-of-heterozygosity (LOH), suggesting recombination events between the alleles. By analysing available genomic sequencing data of other Lister 427 clones, we observed that most of the LOH were conserved, but some were present only in clones adapted to the procyclic lifecycle stage. We also found that some Lister 427 clones were aneuploid. We found evidence of triploidy in Chr5, Chr2 and Chr6, and Chr2 and Chr7. Moreover, by analysing RNA-seq data, we observed that the transcript level is proportional to the ploidy, implying the lack of a general dosage compensation mechanism in *T. brucei*.

Finally, by comparing the proteome from our Lister 427 genome assembly to the one of TREU 927 isolate, we identified a set of genes that are much larger than previously predicted.

We believe that the availability of the fully phased genome of the Lister 427 isolate will, on one hand, allow the analysis of allele-specific mechanisms, so as to better study recombination processes and coevolution, and, on the other hand, serve as a standard to ‘benchmark’ much needed automatic genome assembly pipelines for highly heterozygous trypanosome field isolates.

232C Investigating the role of Plasmodium falciparum exported proteins that bind the new permeability pathway complex protein RhopH2 *Thorey Jonsdottir*1,2, Natalie Counihan3, Benjamin Dickerman1, Smitha Sudhakar3, Betty Kouskousis1,4, Joyanta Modak3, Mikha Gabriela1,3, Paul Sanders1, Hayley Bullen1, Tania de Koning-Ward3, Brendan Crabb1,2,5, Paul Gilson1 1) The Burnet Institute, Melbourne, Australia; 2) The Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne, Australia; 3) School of Medicine, Deakin University, Waurn Ponds, Australia; 4) Monash Micro-imaging, Monash University, Melbourne, Australia; 5) Department of Microbiology, Monash University, Australia.
The ability of \textit{Plasmodium falciparum} to survive within the red blood cell (RBC) is dependent on the activity of the new permeability pathways (NPPs). The NPPs allow import of vital substrates such as isoleucine and pantothenic acid from the blood plasma as the parasite is incapable of making them de novo. These nutrient channels have been affiliated with the activity of three parasite proteins: CLAG3 (Rhoph1), Rhoph2 and Rhoph3, which form a complex at the RBC surface. We previously showed that Rhoph2 was associated with \textasciitilde30 additional proteins predicted to be exported into the RBC. We therefore hypothesised that some of these exported proteins might be important for NPP function, as previous studies have shown that the PTEK machinery, which exports these proteins, is required for NPP activity. Here we studied 14 of the exported proteins found to associate with Rhoph2 and their potential NPP-related functions. Reciprocal binding experiments indicated only four proteins were able to be strongly bind Rhoph2 with the other proteins being weak binders or indirectly associated with Rhoph2. All four proteins belong to the PHISTb protein family, where two have been implicated to have cytoskeletal roles. Indirect immunofluorescence assay showed the four proteins partially co-localise with the NPP protein Rhoph3 at the RBC surface, however, a conditional knockdown of the proteins by 50\% revealed that none were required for NPP activity. This raised the questions as to whether 1) knockdown of each protein was insufficient to reduce NPP activity, 2) if the PHISTb proteins were functionally redundant with respect to NPP activity or 3) the proteins have roles unrelated to the NPPs. With respect to the last point, we note that only two of the PHISTb proteins were found to associate with CLAG3 and Rhoph3 suggesting Rhoph2 and the other two proteins may have additional roles. Deciphering the individual components necessary for NPP activity could inform future drug discovery as blocking these channels would deprive the parasites of essential nutrients thereby arresting their growth. This study also greatly expands our current knowledge of exported proteins and reveals for the first time the location of six new proteins, hypothesised to be exported, one of which was retained within the parasitophorous vacuole. Reciprocal binding assays also reveal several potential new protein complexes giving a clearer picture of functional parasite protein networks within the parasitised RBC.

\textbf{233C Preliminary characterization of myosin D from} \textit{Plasmodium falciparum} \textit{Andrea Johana Lopez Moreno1, Inari Kursula1, 2 1) University of Bergen, Bergen, Norway; 2) Biocenter Oulu and Faculty of Biochemistry and Molecular Medicine, University of Oulu.}

Malaria is a parasitic infection caused by \textit{Plasmodium} spp. In 2019, approximately 405,000 people died from nearly 228 million malaria cases registered worldwide. These parasites display a unique mode of cell motility called gliding motility. A macromolecular motor complex, the glideosome, is indispensable for parasite locomotion and host cell infection. The core of the glideosome is formed by an actomyosin motor comprised of actin, myosin A, and two light chains ELC and MTIP. \textit{Plasmodium} spp. have six myosins classified into three classes (VI, XII, and XIV). Myosin A from class XIV is the most studied. Currently, molecular and structural information is not available for the parasite myosins in class VI. Our goal is to characterise class VI Myosin D (\textit{PfMyoD}) from \textit{Plasmodium falciparum} and to identify the specific light chains for \textit{PfMyoD}.

In the parasites myosin chaperones \textit{PfJUNC45} and \textit{PfHsp90}, the co-expression of light chains promote the proper folding of myosins. However, the correct light chains for \textit{PfMyoD} are not known. A polyprotein approach was used to identify the specific light chains for \textit{PfMyoD}. The polyproteins are long polypeptides, which contain a Tobacco etch virus Nla protease and individual proteins of interest, spaced by proteolytic cleavage sites. There are 11 putative myosin light chains identified in \textit{P. falciparum}. Three polyproteins that contain the light chains were designed and co-expressed with \textit{PfMyoD} and myosin chaperones \textit{PfJUNC45} and \textit{PfHsp90}.

We found that \textit{PfMyoD} co-expressed with two of the polyproteins was soluble. The result was confirmed by mass spectrometry. Co-expression with the polyproteins allows us to get soluble \textit{PfMyoD} for future biochemical and structural experiments. We are in the process to identify the specific light chains for \textit{PfMyoD}.

\textbf{234C Loss of fat mass during a} \textit{Trypanosoma brucei} \textit{infection prolongs host survival} \textit{Henrique Machado1, Peter Hofer2, Rudolf Zechner2, Luisa M. Figueiredo1 1) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; 2) Institute of Molecular Biosciences, University of Graz, Graz, Austria.}

In mammals, the adipose tissue (AT) plays a central role in energy homeostasis and exerts important endocrine functions. The unicellular parasite \textit{Trypanosoma brucei}, a causative agent of African trypanosomiasis, has recently been described to adapt and accumulate in high number in the adipose tissues of infected mice. Here, we investigated how the AT changes during a \textit{T. brucei} infection and how this impacts disease progression. Using a combination of physiological, biochemical and imaging approaches, we show that during a \textit{T. brucei} infection there is a progressive reduction of adipocyte area and AT weight concomitant with an increase of AT lipolysis. Knock-out mice with deficient AT lipolysis present a higher parasite burden in this tissue. Interestingly, these mice are protected from early loss of fat mass but lose more lean mass, succumbing earlier to infection than wild-type controls.

This study shows that AT lipolysis protects the host during a \textit{T. brucei} infection and suggests that the cachexia phenotype typical of African trypanosomiasis may be partially due to the presence of parasites in the AT.

\textbf{235C Involvement of the exported FIKK kinases of} \textit{P. falciparum} \textit{in modulating the cytoadhesive properties of the host cell} \textit{Heledd Davies1, Hugo Belda1, Malgorzata Broncel1, David Jones1, Jill Dalimot1, Prince Nyarko2, Gordon Awandare2, Moritz Treeck1 1) The Francis Crick Institute, London, UK; 2) West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Accra, Ghana.}

\textit{Plasmodium falciparum} exports proteins into the host red blood cell to modify its properties and evade immune detection. Proteins from the PFEMP1, RIFIN and STEVOR protein families are presented on the red blood cell surface and adhere to receptors on endothelial cells and other blood cells, mediating cytoadhesion and rosetting of the infected cell. This can lead to vascular blockages and cause severe malaria. We have previously identified an exported kinase from the FIKK family to be involved in the presentation of PFEMP1 on the cell surface and its binding to Chondroitin Sulphate A (CSA). Here we present data on additional FIKK kinases mediating cytoadhesion and their role in adhesion to receptors other than CSA. Using TurbidID fusion proteins of two kinases targeted to the periphery of the RBC, we start to understand their most likely direct targets, giving insights into how this kinase family may regulate cytoadhesion.

\textbf{236C Iron storage in} \textit{Toxoplasma gondii} \textit{requires} \textit{VIT1} \textit{Clare Harding1, Dana Aghabi1, Alfredo Guerraz2 1) University of Glasgow; 2) University of Michigan.}

Iron is an essential nutrient for all living organisms. Although essential, when in excess, iron can be toxic. Thus, the uptake and sequestration of iron is essential and is carefully regulated in most organisms. As \textit{Toxoplasma gondii} infects all nucleated cells and tissue types during its life cycle, it requires metabolic flexibility to rapidly adapt to a wide range of iron concentrations between tissues, implying that uptake and storage of iron is regulated in the parasite. However, very little is known about iron import, transport and storage within \textit{Toxoplasma gondii}. In yeast and plants, iron is sequestered into membrane-bound vacuoles, mediated by the action of members of the vacuolar iron transporter (VIT) family of proteins. Both \textit{Plasmodium} spp. and \textit{T. gondii} encode a homologue of this transporter, suggesting that apicomplexan parasites may be storing iron in a similar manner.

In \textit{T. gondii}, VIT1 localised to the vacuolar compartment (VAC), suggesting that excess iron may be stored within this lysosomal-like compartment. Upon deletion of VIT1, we observed a mild phenotype under normal growth conditions as compared to the parental line. However, in the absence of VIT1, parasites had excess cytoplasmic iron and upon exposure to excess iron, \textit{ΔVIT1} parasite growth was significantly impaired. These results suggest that VIT1 is...
required for iron storage within the parasite and plays a major role in iron detoxification and the development of *Toxoplasma gondii* within their mammalian host.

**237C Golgi or no Golgi: How do proteins choose between transport pathways to the apicoplast in *Toxoplasma gondii?*** Aparna Prasad1, Pragati Mastud1, Swati Patankar1 1) Indian Institute of Technology Bombay.

The apicoplast of *Toxoplasma gondii* is a secondary endosymbiont, having four membranes, like in other apicomplexan parasites. Its structure is supported by a complex cell biology and unknown mechanisms. One such mechanism is protein transport to the apicoplast. Apicoplast proteins are nuclear-encoded and trafficked to the organelle through the endoplasmic reticulum (ER). From the ER to the apicoplast, two distinct protein trafficking pathways can be used. The proteins could reach the apicoplast either through the cell’s secretory pathway involving the Golgi or through a unique Golgi-independent pathway.

The Golgi-independent and -dependent pathway have been reported using various experimental approaches in both *Toxoplasma gondii* and *Plasmodium falciparum*. This raises questions on the molecular features that drive proteins to each pathway, which is still unknown. We have systematically tested 8 proteins localised to the apicoplast, using a C-terminal HDEL sequence to assess the role of the Golgi in their transport. We demonstrate that dually localised proteins of the apicoplast and mitochondrion *(TgSOD2, TgTPx1/2 and TgACN/IRP)* are trafficked through the Golgi while proteins localised exclusively to the apicoplast are trafficked independently of the Golgi. Further, mutants of dually localised proteins that localised exclusively to the apicoplast also showed trafficking through the Golgi. Phylogenetic analysis of *TgSOD2, TgTPx1/2* and *TgACN/IRP* suggested that the evolutionary origins of *TgSOD2, TgTPx1/2* lie in the mitochondrion while *TgACN/IRP* appears to have originated from the apicoplast. With these results, we propose a model that dual localised proteins of the apicoplast and the mitochondrion exclusively employ a unique Golgi-dependent pathway to the apicoplast while exclusively apicoplast localised proteins are trafficked independent of the Golgi.

**238C The single CCA-adding enzyme of *T. brucei* has distinct functions in the cytosol and in mitochondria** Shikha Shikha1, Andre Schneider2 1) Wellcome Centre for Integrative Parasitology, Glasgow; 2) Department of Chemistry and Biochemistry, University of Bern.

All tRNAs contain a three nucleotide C-terminal CCA tail, required for tRNA aminoacylation and correct positioning of the tRNAs in the ribosome. In all the eukaryotes, the CCA tail is added post-transcriptionally by an enzyme called the CCA-adding enzyme. The single mitochondrial of the parasitic protozoan *Trypanosoma brucei* lacks tRNA genes and therefore imports all of its tRNAs from the cytosol. This presents a unique situation for tRNA modifying activities in and outside the mitochondrion of *T. brucei*. Here, we identified a single ORF, which we called *TbCAE*, as a potential candidate for encoding the CCA-adding enzyme in *T. brucei*. Knockdown of *TbCAE* led to a swift growth defect, accumulation of truncated tRNAs, abolishment of protein synthesis and inhibition of both cytosolic and mitochondrial CCA-adding activities, indicating that *TbCAE* is located both in the cytosol and mitochondrion. However, mitochondrial tRNAs were much less affected by *TbCAE* ablation compared to the cytosolic ones. Complementation with the corresponding yeast enzyme showed that the enzyme does not need the N-terminal 10 amino acids for its mitochondrial localisation or activity. Furthermore, the growth defect caused by the knockdown of endogenous *TbCAE* could be rescued by the expression of cytosolic isoform of yeast CAE, indicating that the essential function of TbCAE for adding the CCA tail to the primary tRNA transcripts, is in the cytosol. In fact, ablation of the mitochondrial TbCAE activity, which likely provides repair function, only marginally affected the parasite growth.

**239C Toxoplasma FER1 Mediates Trafficking and Discharge of the Micronemes** Allison Drozd1, Daniel Tagoe1, Bradley Coleman1, Isabelle Coppens2, Marc-Jan Gubbels1 1) Biology Department of Biology, Boston College, Chestnut Hill, MA; 2) Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

The lytic life-cycle of the parasite *Toxoplasma gondii* is driven by calcium-dependent processes of host cell invasion and egress. Secretion of apically localised organelles, the micronemes, precedes the events of invasion and egress. Likewise, secretion of rhoptry proteins has also been shown necessary for invasion. While much of the signaling pathway leading to microneme secretion is known, open questions regarding microneme trafficking and membrane fusion remain. Mammalian Ferlin family proteins, one of which, FER2, has already been shown necessary for rhoptry secretion. We hypothesized that *Toxoplasma* FER1 is involved in trafficking micronemes and mediating membrane fusion for secretion, similar to its mammalian counterparts. Using a conditional overexpression system, FER1 prematurely triggers microneme secretion and subsequent egress. The TEM ultrastructure provided further insight as to where overexpression causes mis-localization and accumulation of micronemes at the apical tip. To address the potential for calcium-dependency, we used site-directed mutagenesis to mutate calcium stabilizing residues within the C2 domains. Subsequently we assessed microneme localisation via immunofluorescence. Mutating the calcium binding pocket of FER1 differentially affected different microneme sub-populations, whereby some populations were diminished at the apical tip and others seemed unaffected. These results indicate that FER1 plays a role in trafficking micronemes to the apical tip as well as their secretion.

**240C Glutamine is an important metabolite for *T. cruzi* to survive throughout the life cycle** Flávia Damasceno1, Rodolpho Souza1, Sabrina Marsiccobetre1, Ariel Silber1 1) University of São Paulo - USP.

*Trypanosoma cruzi* is the causative agent of American trypanosomiasis, also known as Chagas disease. *T. cruzi* life cycle alternates between the mammalian host, among them the humans, and a reduvid insect vector. Inside both host *T. cruzi* differentiates among several stages: amastigote, intracellular epimastigote and trypomastigote in the mammalian host, and epimastigotes and metacyclic trypomastigote in the insect vector. As *T. cruzi* needs to colonize different habitats, such as, the bloodstream and cells cytoplasm in the mammalian host, and the intestine in the insect vector, it faces environments with different nutrient compositions. In our work we show the important role of Glutamine (Gln) in the parasite survival in the different environments during its life cycle. *T. cruzi* can obtain Gln by its uptake from the external medium or can synthesize it from glutamate, ATP and NH4+ as substrates. The parasite uses alternately both complementary sources of Gln through the life stages of the parasite. Moreover, our data shown that Gln is a relevant metabolite to many stages of adaptation of *T. cruzi* according to nutrient availability.
fluorescence microscopy of genetically engineered parasites, we demonstrate that FT2 localizes to the apicoplast. In invasive *P. berghei* stages, a fraction of FT2 is also observed at the apical end. Upon genetic disruption of FT2, blood and liver infection, gametocyte production, and mosquito colonization remain unaltered. But in the *Anopheles* vector, FT2-deficient parasites develop inflated oocysts with unusual pup formation consisting of numerous single-membrane vesicles, which ultimately fuse to form large cavities. Ultrastructural analysis suggests that this defect reflects aberrant sporoblast formation caused by abnormal vesicular traffic. Complete sporogony in FT2-deficient oocysts is very rare, and mutant sporozoites fail to establish hepatocyte infection, resulting in a complete block of parasite transmission. Our findings reveal a previously unrecognized organelar folate transporter that exerts critical roles for pathogen maturation in the arthropod vector.

**242D Same parasite - different coats: A case of surface functionality transfer between trypanosome species**

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An Alba-domain protein required for proteome remodelling during trypanosome differentiation and host transition

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Trypanosoma brucei, a unicellular eukaryotic parasite, needs two hosts, mammals and tsetse flies, in order to complete its life cycle. Throughout its developmental cycle, *T. brucei* encounters diverse environments to which it has to adapt in order to maintain its transmission and infectivity. Successful
adaptation to the new environment and transition to different life-cycle stages are the general challenges faced by many digenetic parasites. In this study we show that the Alba-domain protein Alba3 is essential for differentiation of the mammalian stumpy form (transition form) to the procyclic form in the tsetse host. An Alba3 deletion mutant infects mice and shows characteristic waves of parasitaemia, but is severely compromised in its ability to infect tsetse flies. Stumpy forms are translationally repressed, but are poised to resume protein synthesis during differentiation. We show that Alba3 is key to escape from translation repression; in its absence, there is a delay in the formation of polysomes and resumption of protein synthesis. While steady state levels of mRNAs in differentiating cells are barely affected by the loss of Alba3, there are major repercussions for the proteome. This impacts the formation of procyclic-specific mitochondrial respiratory complex proteins as well as the repression of some bloodstream-specific proteins. Interestingly, Alba3 and the closely related Alba4 are functionally redundant in slender forms, but Alba4 cannot compensate for the lack of Alba3 during differentiation from the stumpy to the procyclic form. This is the first time that a single protein has been shown to have a major influence on translation as an adaptive response to changing hosts. It is also the first time that a mechanism has been established for Alba-domain proteins in parasites. We postulate that Alba-domain proteins play similar roles in regulating translation in other protozoan parasites, in particular during life-cycle and host transitions.

247D Novel telomere-associated proteins and their impact on VSG expression site regulation in Trypanosoma brucei Nadine Weisert1, Helena Reis1, Marie Schwebs1, Katarina Luko2, Mario Dejung2, Falk Butter2, Christian Janzen1 1) Universität Würzburg; 2) Institut für molekulare Biologie (IMB) Mainz.

Trypanosoma brucei is the causative agent of African trypanosomiasis, also known as African sleeping sickness. The survival of the extracellular parasite in the mammalian host is based on mono-allelic expression of a variant surface glycoprotein (VSG) coat.

T. brucei encodes a large library of VSG genes, but only one is ever expressed at a time. This single VSG gene is transcribed from one of 15 expression sites (ES). The ES are all located at subtelomeric regions of chromosomes, suggesting that telomere-associated proteins may contribute to transcriptional regulation of VSG expression. Previously characterized telomeric proteins such as TbTRF, TbTFI, and TbRAP1 have been shown to play a role in subtelomeric VSG gene regulation, but we assume that the identification of all telomeric protein complexes has not yet been achieved. To fully understand how telomere-associated proteins (TelAPs) contribute to VSG regulation, it is essential to discover novel telomeric proteins.

Two complementary biochemical approaches were used to identify novel TelAPs. First, affinity chromatography with telomeric repeat oligonucleotides was used to pull down TelAPs from trypanosome lysates. Second, a co-immunoprecipitation with TbTRF, which binds directly to telomeric repeats, was used to identify interaction partners. 24 potential TelAPs were significantly enriched by these approaches.

Interestingly, one of the candidates, PolIE, is a protein with a dual function. Previously, it has been demonstrated that RNAi-mediated depletion of PolIE results in slowed growth, altered DNA content, changes in cell morphology, and increased sensitivity to DNA damaging agents. In addition, we now show that PolIE depletion leads to deregulation of VSG genes, linking the function of this translesion DNA polymerase to host immune evasion by antigenic variation. The role of other novel TelAPs in antigenic variation and the possibility of stage-specific telomere complex composition will also be discussed.

248D Antileishmanial chemotherapy through clemastine fumurate mediated inhibition of the Leishmania inositol phosphorylceramide synthase Edubiel Alpicar1, John Mina2, Rebecca Charlton4, Douglas Escrivan1, Emily Dickie3, Wenbin Wei1, Andy Merritt5, Terry Smith6, Michael Barrett3, Bartira Rossi-Bergmann4, Paul Denny1, Patrick Steel2 1) Department of Biosciences, University of Durham; 2) Department of Chemistry, University of Durham, Science Laboratories; 3) Wellcome Centre for Integrative Parasitology, University of Glasgow, Glasgow; 4) Institute of Biophysics, Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil; 5) LifeArc, Open Innovation Campus; 6) BSRG, Schools of Biology and Chemistry, University of St Andrews.

Current chemotherapuetics for leishmaniasis have multiple deficiencies and there is a need for new, efficacious and affordable medicines. This study describes a successful drug repurposing approach that identifies the over-the-counter antihistamine, clemastine fumarate as a potential antileishmanial drug candidate. Screening for inhibitors of the inositol phosphorylceramide synthase (IPCS) afforded, following secondary screening against Leishmania major promastigotes, 16 active compounds. Further refinement through dose response against LmjIPCS and intramacrophage L. major amastigotes identified clemastine fumarate, with good activity and selectivity with respect to the host macrophage. On target engagement was supported by diminished sensitivity in a sphingolipid-deficient L. major mutant (ΔLmjLCB2) and altered phospholipid and sphingolipid profiles upon treatment with clemastine. The drug also induced an enhanced host cell response to infection indicative of polypharmacology. Activity was sustained across a panel of Old and New World Leishmania species, displaying in vivo activity equivalent to the currently used drug, glantucine, in a mouse model of L. amazonensis infection. Interestingly, selection for resistance to clemastine generated mutants with variants (SNPs) in eight genes of the sphingolipid pathway in L. major and L. mexicana. This was consistent with the changes observed in abundance of intermediates involved in sphingolipid metabolism measured using targeted metabolomics after exposure to clemastine. Significantly, for a potential new antileishmanial chemotherapy, clemastine displays low tendency to generate highly resistant promastigotes with no evidence for cross-resistance with the currently used frontline antileishmanial drugs. Overall, these data indicate clemastine fumarate to be a strong repurposing candidate for the treatment of leishmaniasis.

249D New insights into Trypanosoma brucei chromatin dynamics Tim Vellmer1, Katharina Luko2, Falk Butter2, Christian Janzen1 1) University Wuerzburg; 2) Institute for Molecular Biology (IMB), Mainz, Germany.

Chromatin dynamics in the unicellular parasite Trypanosoma brucei, the causative agent of human African trypanosomiasis, regulate processes that are essential for the parasite’s survival. For example, transcriptional regulation of the variant surface glycoprotein (VSG) genes, which allows the parasite to evade the host immune system, is influenced by changes in chromatin structure. Despite the importance of these processes, they are still far from being completely understood.

Nucleosomes are subjected to various remodeling processes during the cell cycle, thus they represent the basis for chromatin dynamics. New nucleosomes have to be assembled during DNA replication, and already existing nucleosomes have to be repositioned or modified to enable specific processes. Accurate transcription of the polycistronic transcriptional units (PTUs), requires the correct incorporation of the histone variants H2AZ, H2BV, H3V and H4V at specific sites that flank the PTUs. Although some posttranslational histone modifications that regulate these processes have been described, the machinery that exchanges core histones with their corresponding variants, still remains elusive. Conserved protein complexes such as INO80, or SWR1, that are known to be especially involved in the exchange of H2A and its variant in other taxa, could not be identified in T. brucei so far.

During proximity labelling experiments that aimed to provide insight into the replication regulation by the lysine methyltransferase DOT1A, we identified components of a potential new chromatin remodeling complex. A subsequent co-immunoprecipitation (Co-IP) experiment identified an essential protein that belongs to the SNF2 superfamily. It interacts with several other proteins including actin, actin related proteins, a YEATS- and a YL1-domain containing protein. In silico analysis of all identified proteins, in combination with co-purification of SWR1-specific factors suggest that this newly found complex is indeed a SWR1-like chromatin remodeler.

Follow up experiments including Co-IPs, chromatin immunoprecipitation (ChIP) and cell fractionations provided further information about the exact composition of the complex, and its relevance for H2AZ incorporation into chromatin.
Trypanosoma cruzi is the etiologic agent of Chagas disease, which is transmitted by triatomine insects. As the life cycle of T. cruzi needs an invertebrate vector and a vertebrate host to completion, this parasite is highly adapted to different environments. For example, when glucose is limited in the insect vector’s gut, T. cruzi can degrade amino acids producing ammonia. Histidine (His) is present in high concentrations in the insect’s gut and the parasite can use it to generate ATP through its conversion in glutamate. This pathway consists of four enzymatic steps. The coding sequences for the first two enzymes and putative coding sequences for the two last enzymes are present in the T. cruzi’s genome. However, they are absent in Trypanosoma brucei which makes it a useful model to investigate the biological role of this pathway. To express the complete His degradation pathway from T. cruzi in T. brucei, we developed a series of plasmids, named pGIS. We transfected T. brucei procyclic form with the plasmid pGIS001, which allows constitutive expression of histidine ammonia-lyase from T. cruzi (TcHAL). Despite its active expression as shown by western blotting and activity assays, there is no difference in proliferation between the wild-type control and the transfected parasites. Then, we transfected T. brucei procyclic form with the plasmid pGIS018, which allowed the constitutive expression of T. cruzi urocanate hydratase (TcUH), as assayed by Western blotting and measurements of TCuH enzymatic activity in cell-free extracts. When we generated T. brucei procyclic forms bearing plasmids pGIS001 and pGIS018 we obtained parasites constitutively expressing both enzymes, as seen by Western blotting. However, enzymatic activity assays demonstrated that only one of the six selected clones have a detectable specific activity for both enzymes. In the enzymatic assays for TcHAL and TCuH we measured, respectively, the formation and degradation of urocanate, therefore variations in the expression levels of both enzymes can affect the detection of this metabolite. Analysis of the complete His degradation pathway in T. brucei can contribute to elucidate which evolutionary advantages it brings to T. cruzi’s life cycle and provide valuable insights to the development of drugs that interfere with its metabolism.

251D Lysine residues are critical for heterogeneous accumulation of P. falciparum PCNA1 among nuclei

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Cells typically reproduce by duplicating their daughter and subsequently dividing into two daughter cells. Plasmodium falciparum (P. falciparum) displays a remarkably different mode of reproduction and multiplies through schizogony: consecutive rounds of DNA replication and nuclear division occur, forming a multinucleated cell before daughter cells are assembled. Strikingly, nuclei appear to divide asynchronously during schizogony despite sharing the same cytoplasm. Using live-cell imaging we found that DNA replication also occurs asynchronously in schizonts with multiple nuclei. To investigate the dynamics of replication further, we employed a reporter cell line where nuclei are marked with mCherry and PCNA1 (a component of the DNA replication fork) is fused to eGFP. We found that PCNA1 temporarily accumulates in nuclei, which coincided with a doubling of the DNA content. At a given time PCNA1 accumulates only in a subset of nuclei, providing further evidence that DNA replication occurs asynchronously. However, the mechanistic basis of how proteins accumulate heterogeneously among nuclei that reside in the same cytoplasm is unclear. To investigate this, we first assessed if nuclei are indeed discrete units or whether they are interconnected using serial-section electron tomography. We found that nuclei are independent cellular compartments, which reside in very close proximity (<100 nm). To inform on the mechanistic basis of heterogeneous PCNA1 accumulation, we next analyzed a series of PCNA1 mutants. Our data indicate that the nucleocytoplasmic transport of PCNA1 is active, highlighting the importance of this process in the context of asynchrony. In addition, we found that multiple lysine residues are critical for heterogenous nuclear accumulation of PCNA1. Together, our data suggest that both DNA replication and nuclear division occur asynchronously and that nuclei independently control their protein content, which is key to the high degree of autonomy of P. falciparum nuclei.

252D Expansion microscopy of TAC protein-complex arrangement in T. brucei

Ana Kalichová, Simona Amadeo, Laura Pfeiffer, Bernd Schimanski, Andre Schneider, Torsten Ochsenreiter 1) University Bern.

Trypanosoma brucei contains a single, tubular mitochondrial organelle with a uniquely organized mitochondrial DNA called kinetoplast DNA (kDNA). The kDNA consists of catenated mini- and maxicircles that together form a disk-like network which is physically linked to the basal body. The structure mediating the linkage is termed the tripartite attachment complex (TAC). The TAC spans three regions in the cell and currently more than 10 different proteins have been associated to that machinery. In order to assess the dynamics of TAC assembly, I have established Ultrastructure expansion microscopy (U-ExM) that allows for isotropic expansion of T. brucei cells by a factor of four to five. By applying U-ExM we are able to overcome optical limitation of resolution problem in microscopy field. Here, I present results on the evaluation of U-ExM in trypanosomes and TAC component localization relative to each other and the kDNA.

253D Parasitophorous vacuole morphology and modulation of pH at the time of Plasmodium falciparum egress from erythrocytes


To egress from its host red blood cell, the malaria parasite Plasmodium falciparum breaches two surrounding membranes, the vacuolar (PVM) and erythrocyte membrane, in a highly orchestrated multi-step event. Following the natural parasite egress sequence under a fluorescence microscope using endogenously mNeonGreen-tagged vacuolar membrane resident protein EXP2, we made two curious observations: (1) signal from the EXP2 was found intruding far into the segmented parasite, and (2) the fluorescent signal of EXP2 sharply decreases at the moment of PVM rupture.

We hypothesize that both observations may be related. The intrusion may act as a path that can connect the acidic compartments inside the parasite, such as digestive vacuole (DV), to the vacuolar lumen and RBC cytoplasm. A subsequent drop of pH in the PV and the red cell could be a part of the egress mechanism. Using focused ion beam scanning electron microscopy (FIBSEM) and conventional thin-section EM we found that indeed the RBC cytosol intrudes deep into the segmented parasite in tubular extensions of the PVM. The tubular extensions have been observed in a cytoskeleton-like structure frequently contacting with the digestive vacuole. Next, to assess the requirement of acidification for parasite egress, schizonts were treated with weak bases and an ionophore to increase or dissipate the pH in acidic organelles. We found that 30 min acute treatment of schizonts with ammonium chloride, chloroquine and monensin impedes the parasite to finish its egress process.

We conclude that pH may play a role in parasite egress mechanism and that PVM tubules offer a putative delivery pathway to acidify the intracellular environment of the parasite.

254D The serine-cysteine interconversion is catalyzed by serine acetyltransferase and cysteine synthase in Trypanosoma cruzi

Ana Milena Murillo Giraldo, Sabrina Marsicocchette, Ariel M Silber 1) Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo.

Trypanosoma cruzi can use amino acids as energy sources and to support several biological processes such as differentiation, resistance to stress conditions and host-cell invasion. Metabolites containing -SH groups (such glutathione, trypanothione, cysteine, and some of its intermediates such as cystathionine) are relevant to buffer the redox state of the different sub-cellular compartments of this organism. The de novo cysteine biosynthesis pathway is comprised of serine O-acetyltransferase (SAT) and cysteine synthase (CS) enzymes which sequentially mediate two consecutive steps of cysteine biosynthesis,
Ariel M Silber
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resulted in the lack of crotonase activity as confirmed by measuring the enzyme activities in cell-free extracts. The absence of the enzyme did not affect the evaluate the role of this enzyme in the parasite biology, we obtained knock out mutants for both copies of Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana; 2) Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

These conditions starved supernatants and also identified the putative genes encoding TcSAT and TcCS enzymes, which were cloned, expressed, and affinity-purified. The recombinant TcSAT and TcCS (which contains PLP as cofactor) predicted the molecular mass 38 kDa and 36 kDa, respectively. The biochemical characterization showed that TcSAT catalyzes the synthesis of O-acetylspermine, whereas TcCS catalyzes the cysteine formation using OAS and one of the S–H. The Km 0.2566 mM and 3.9 mM for the recombinant proteins were determined for Serine and OAS, and its Vmax was around 0.33 µmol/min/µg and 2.8 mol/min/µg, respectively. Additionally, we obtained specific polyclonal antisera against both recombinant enzymes. We obtained knock out mutants in the CL Brener strain by the CRISPR-Cas9 methodology for both genes to evaluate the role of these enzymes and determined the important role that this pathway have in the redox state.

255D Effects of Trypanocidal drugs on DNA synthesis: insights into the mode of Killing of Melarsoprol Stephen Larson1, McKinzie Carter1, Galadriel Hovell-Miner1 1) George Washington University.

Thiol redox in Trypanosomatids predominantly relies on trypanothione [bis(glutathionyl)spermidine]. The biosynthesis and utilization pathways for trypanothione are characterized by Trypanosomatid specific enzymes and molecular substances that make ideal drug targets against the human parasites in this family. The drug melarsoprol inhibits trypanothione function through direct binding, which inhibits downstream redox biology with anticipated effects on thiol redox, ROS management, and dNTP synthesis. The diverse outcomes of trypanothione inhibition has made elucidating the specific mode of cell killing of melarsoprol a challenge. Because the trypanothione pathway is required for the function of ribonucleotide reductase to generate dNTPs, we hypothesized that melarsoprol treatment would inhibit DNA synthesis. To test this hypothesis we analyzed the cell cycle and DNA synthesis defects arising from trypanocidal drugs with known connections to the trypanothione pathway or DNA damage. We found that DNA synthesis is specifically inhibited by melarsoprol treatment and not the other trypanocidal drugs tested. Overexpression of the rate-limiting enzyme of trypanothione biosynthesis (g-glutamylcysteine synthetase) was able to partially alleviate the melarsoprol induced DNA synthesis defect as well as increasing survival in the presence of drug. These findings implicate DNA synthesis as a possible mode of cell killing for melarsoprol.

256D Participation of an Enoyl-CoA hydratase in the catabolism of branched chain amino acids in Trypanosoma cruzi Sabrina Marsicobotte1, Ana M Murillo1, Ariel M Silber1) Laboratory of Biochemistry of Tryps-LaBTryps, Department of Parasitology, Institute for Biomedical Sciences, University of São Paulo.

Amino acids have numerous functions in T. cruzi beyond their participation in protein synthesis. The capacity of this parasite to metabolize branched chain amino acids (BCAA – Leu, Ile or Val), and the importance of their metabolic intermediates for the parasite biology are points of our interest. The enoyl-CoA hydratase (ECH) or crotonase, catalyzes the second step of the β-oxidation pathway, and also has been involved in the fourth step in the BCAA metabolism. The sequences that encode for enzymes associated with BCAA catabolism are present in the T. cruzi genome. The branched chain keto acids derived from BCAA are oxidized by the α-keto acid dehydrogenase complex, and dehydrogenated by acyl-CoA dehydrogenases. Subsequently, the products can be metabolized by an ECH, producing acetyl-CoA, which in turn can feed the tricarboxylic acid cycle. In the current work, the coding sequence of ECH of T. cruzi (TcECH) was expressed and purified from Escherichia coli pGRO7. We determined for the recombinant enzyme a Km of 179.8 ± 31.2 µM and a Vmax of 0.034 µM/min/µg using crotonoyl-CoA. Specific polyclonal antibodies were obtained and used to determine its mitochondrial localization in epimastigote forms of the parasite. To evaluate the role of this enzyme in the parasite biology, we obtained knock out mutants for both copies of tcech by using CRISPR/Cas9. The TcECH gene ablation resulted in the lack of crotonase activity as confirmed by measuring the enzyme activities in cell-free extracts. The absence of the enzyme did not affect the parasites proliferation. The knocked out parasites were submitted to nutritional stress by incubation in PBS supplemented or not (control) with Val, Ile or Leu for 96 hours, to assay their ability to survive to these conditions. We observed that parasites maintained in PBS supplemented with Leu or Ile recovered from starvation as well as wild type parasites. However, those supplemented with Val did not recover indicating the accumulation of a toxic metabolic intermediate in these conditions. Finally, we show that TcECH is involved in BCAA metabolism. This will allow us to better understand the role of the BCAA oxidation pathway for ATP production, and thus, for the biology of T. cruzi.

257D Bioactive trypanocidal compounds from a locally used herbal preparation in Ghana Pearl Akazue1,2, Neils Quashie1, Theresa Gwira1 1) West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana; 2) Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

In sub-Saharan Africa, where the most enormous burden of human and animal African trypanosomiasis is felt, the use of herbal treatments is a common practice. These herbal treatments are readily available and sometimes efficacious. With rising levels of resistance to the conventional chemotherapeutic drugs currently in use, there is a great need for new antitrypanosomal compounds that could be used for single or combination therapy. In the present study, six fractions obtained by maceration of dried herbs from locally used herbal preparations in dichloromethane were tested against Trypanosoma brucei brucei GUTat 3.1 strain in vitro. Two of the fractions possessed antitrypanosomal activity with IC50 values of 8.50µg/ml and 7.37µg/ml compared to an IC50 of 0.13µg/ml for Trypanosoma brucei muris. The fractions and mother liquors that showed activity had good selectivity for trypanosomes. Static/cidal assay revealed that the other trypanocidal drugs tested. Overexpression of the rate-limiting enzyme of trypanothione biosynthesis (g-glutamylcysteine synthetase) was able to partially alleviate the melarsoprol induced DNA synthesis defect as well as increasing survival in the presence of drug. These findings implicate DNA synthesis as a possible mode of cell killing for melarsoprol.

VSG Specific Nanobodies Hamper Mobility of the Surface Protein and Lead to Shedding of Nanotubes Laura Hartleb1, Alexander Hempelmann2, Monique van Straaten2, Hamidreza Hashemi3, Johan P. Zeelen2, F. Nina Papavasiliou3, C. Erec Stebbins2, Markus Engstler1, Nicola G. Jones1,1) Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany; 2) Division of Structural Biology of Infection and Immunity, German Cancer Research Center, Heidelberg, Germany; 3) Division of Immune Diversity, German Cancer Research Center, Heidelberg, Germany.

The surface coat of African trypanosomes, which forms the interface between the extracellular pathogen and host, plays an important role in the evasion of host immune responses. At any given time the coat consists predominantly of only one member of the large family of variant surface glycoproteins (VSGs). Trypanosomes side-step clearance by host generated antibodies by repeatedly exchanging their major coat protein through expression of a different member of the large repertoire of VSG genes encoded in their genome.

It has been shown previously that cameldid antibody derived nanobodies raised against VSG AnTat1.1 can bind to the surface coat and cause cell death by a to date unknown mechanism.

In the presented study we analysed the effect of nanobodies raised against VSG2. Crystal structure analysis reveals the binding sites of different nanobodies and mutagenesis studies show differences in their binding affinities to VSG2. This data is presented on an independent poster by the same authors.
Here, we focus on the effects of four different nanobodies on trypanosome viability. An initial screen identified that only one of the four surface binding nanobodies, NB11VS2G, had a strong effect on cell viability. Further studies on this nanobody showed that preceding cell death, cell motility and also VSG mobility on the cell surface were impaired. Interestingly, these effects were associated with the fast formation of long VSG-coated nanotubes, which could be observed within minutes of nanobody addition.

259D Counting cycles or counting on sensing? – Possible mechanisms allowing for P. falciparum persistence during the dry season Carolina Andrade1, Usama Dabbas1, Hannah Fleckenstein1, Nikolay Sergeev1, Safiatou Doumbo2, Jessica Briggs3, Carrie Anderson1, Shanping Li4, Hamidou Cisse2, Didier Doumable2, Muhammad Aghar5, Anna Farnert5, Kassoum Kayenta2, Aissata Ongblove2, Bryan Greenhouse4, Boubacar Traore2, Peter Crompton3, Silvia Portugal1 1) Center of Infectious Diseases, Parasitology, Heidelberg University Hospital, Heidelberg, Germany; 2) Mali International Center of Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali; 3) Department of Medicine, University of California San Francisco, San Francisco, USA; 4) Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA; 5) Division of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet, Stockholm Sweden.

In many areas of the globe, a dry season limits Anopheles mosquito availability, reducing malaria transmission to virtually zero. In Mali, during the 5- to 6-month dry season, Plasmodium falciparum persists in a fraction of the human population in clinically silent infections that serve as reservoir to restart transmission when mosquitoes return. We have recently shown that during the dry season parasites are transcriptionally distinct from those of subjects with febrile malaria in the transmission season, reflecting longer circulation within each replicative cycle, of parasitized erythrocytes without adhering to the vascular endothelium thus promoting improved splenic clearance and sustaining low parasitaemias. The mechanisms leading to longer circulation within each cycle are however still not understood.

To investigate if P. falciparum responds to seasonal factors, we first questioned if persisting parasites in the dry season were able to cause disease in the same individual in the ensuing wet season; and then if plasmas from different times of the year could affect parasite development, replication or cytoadhesion of P. falciparum cultured in vitro, which could seasonally favor subclinical infections. Our data suggests that parasites surviving the dry season do not cause disease in the same individual in the next transmission season. And, thus far, we observe that culturing parasites in different season plasmas does not affect parasite development or replication, and we are currently determining its effects on cytoadhesion and host cell remodelling which could affect parasite clearance. Alternatively to the sensing hypothesis, we questioned if time length of P. falciparum infection in the human host associates with decreased parasite virulence which may also favor subclinical infections towards the dry season. We followed children that remained infected throughout the dry season retrospectively until when they had first become parasite positive in the preceding transmission season, capturing longitudinal data on parasite density fluctuations throughout their infection. Additionally, we will use ampiclon deep sequencing to genotype the parasite clones present at every time point and determine when the parasites present at the end of the dry season first infected the patient. The results from this work will elucidate on the mechanisms that allow P. falciparum to survive the dry season, bridging two transmission seasons.

260D Histidine ammonia-lyase knockout alters bioenergetics of Trypanosoma cruzi without affecting its infection capability in the insect vector Janaina de Freitas Nascimento1, Rodolphe Ornitz Oliveira Sousa1, Leticia Marchese1, Leticia Sophia Silva2, Alessandra Aparecida Guarnieri2, Ariel Mariano Silber1 1) Universidade de São Paulo, São Paulo, SP, Brazil; 2) Instituto René Rachou - Fiocruz Minas, Belo Horizonte, MG, Brazil.

Trypanosoma cruzi, the causing agent of Chagas disease, is highly adapted to life in different environments, from the lumen of the insect vector’s gut to the cytoplasm of cells of the mammalian host. Part of this adaptation is conferred by the metabolic plasticity these organisms have. In this context, amino acids metabolism plays a crucial role in different biological processes that allow T. cruzi to survive, replicate, and progress through its life cycle. Within this framework, our lab has previously characterised the ability of T. cruzi epimastigotes to uptake and fully oxidise histidine (His) to CO2 what points to the existence of an active His degradation pathway in these parasites and to the possible importance of this pathway in parasite persistence within its invertebrate host. In other organisms, the histidine degradation pathway comprises at least four enzymatic steps, being the first the non-oxidative deamination of His to urocanate which is catalysed by histidine ammonia-lyase (HAL). To investigate the biological roles of the putative TcHAL, we generated T. cruzi CL Brener epimastigotes TcHAL null mutants using CRISPR-Cas9 technology. TcHAL null mutants showed no proliferation phenotype when compared to the wild type and Cas9-expressing controls. When TcHAL null parasites were incubated with [14C(U)]-His, they did not produce considerable levels of radiolabelled CO2, showing the successful disruption of the His degradation pathway. Differently from Cas9-expressing controls, when TcHAL null mutants were recovered from nutritional stress with His, they were not able to use His to trigger oxygen consumption. However, recovery of TcHAL null mutants from starvation with urocanate, the immediate product of an active His degradation pathway in these parasites and to the possible importance of this pathway in parasite persistence within its invertebrate host. Part of this work will elucidate on the mechanisms that allow P. falciparum to survive the dry season, bridging two transmission seasons.

261D New approach for understanding the interface between host antibody and Variant Surface Glycoprotein in Trypanosoma brucei using Phage Immunoprecipitation Sequencing Bailin Zhang1, Alexander Beaver1, 2, Daniel Monaco2, Stephanie Henson2, Benjamin Larman2, Monica Mguine1 1) Johns Hopkins University, Bloomberg School of Public Health; 2) Johns Hopkins University, School of Medicine, Department of Pathology.

Trypanosoma brucei, an entirely extracellular protozoan parasite, resides in the blood and tissues of its mammalian host. It adeptly evades host immune recognition via antigenic variation, in which the parasite periodically switches expression of its Variant Surface Glycoprotein (VSG) coat, using a genomic repertoire of ~ 2,000 VSG genes. The parasite’s sophisticated switching mechanism and massive archive of VSG render the host’s immune response ineffective, although antibodies are generated against each VSG during infection. Despite the fact that the antibody response to VSG has been studied extensively, the interaction between antibody and VSG remains poorly understood. Here, we study the interface between antibody and VSG during T. brucei mouse infections using Phage Immunoprecipitation Sequencing (PhIP-Seq). PhIP-Seq is a high throughput epitope mapping approach that utilizes phage display to screen serum recognition of up to thousands of peptides at once. In our initial PhIP-Seq experiments, we found several immunodominant epitopes in VSG3 that are recognized in multiple independent infections. Interestingly, these epitopes, which are 10 times more likely to be targeted by host antibody than other VSG peptide epitopes in our library, are primarily found in the C-terminal domain (CTD) of VSG. As we are currently expanding upon these experiments to determine whether the CTDs of other VSGs display similar immunodominance. The data presented here provide new insights into antibody recognition of VSG, which could inform the design of future T. brucei vaccines or diagnostics.

262D Similarities and differences of metacyclic to bloodstream Variant Surface Glycoprotein (VSG) in Trypanosoma brucei Monica Chandra1, Elaine Thion3, Lucy Glover3, C. Erec Stebbins2, F. Nina Papavasiliou1 1) Division of Immune Diversity, German Cancer Research Center, Heidelberg, Germany; 2) Division of Structural Biology of Infection and Immunity, German Cancer Research Center, Heidelberg, Germany; 3) Trypanosome Molecular Biology Group, Institut Pasteur, Paris, France.
**Trypanosoma brucei** (*T. brucei*) is a unicellular pathogen and the causative agent of African sleeping sickness in sub-Saharan Africa. It is transmitted from one mammalian host to another by the tsetse fly (*Glossina spp.*). The parasite’s surface is densely covered by Variant Surface Glycoproteins (VSGs), which are GPI-anchored. There are approximately 2,000 different VSG genes and pseudo-genes present in the *T. brucei* genomic repertoire. The vast number of VSG genes enables the parasite to repeatedly change the VSG expressed on its surface into an antigenically distinct VSG to evade the immune response (antigenic variation). VSGs are expressed in two life stages of *T. brucei*, the bloodstream and metacyclic stages. The former swims freely in the bloodstream of the mammalian host, and the latter inhabits the salivary gland of the tsetse fly and is transmitted into the mammalian host when the fly takes a bloodmeal. A specific subset of five different VSGs is expressed in the metacyclic form (mVSGs) of *T. brucei* strain Listen427. This study focuses on comparing the bloodstream VSGs and mVSGs on structural level. We have successfully purified and determine the structure of mVSG531 purified from bloodstream *T. brucei* expressing mVSG531. The overall fold and structure of mVSG531 reveals similarities with the structures of bloodstream VSG1 (Mitat1.1) and VSG2 (Mitat1.2), while sharing low sequence identity with these proteins. However, the antisera elicited in C57Bl/6j mice by bloodstream *T. brucei* expressing mVSG531 fails to recognize mVSG531 expressed on the surface of the metacyclics differentiated from procyclics. Meanwhile, transcriptomic data clearly shows that mVSG531 is expressed on the surface of the metacyclics differentiated from procyclics. This suggests either a conformational difference or a post-translational modification of mVSGs when expressed in their proper life stage. Studying the protein structures of different VSGs from different life stages of *T. brucei* will give us a better understanding of the function of VSG in different life stages, as well as antigenic variation in *T. brucei*.

**263D Characterizing the intracellular niche of human microsporidian parasite Encephalitozoon intestinalis**

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Microsporidia are a group of obligate intracellular pathogens related to fungi that cause lethal opportunistic infections in immunocompromised patients including AIDS patients. As such, microsporidia have evolved highly reduced genomes, having lost most protein coding genes for metabolic pathways and co-opt metabolites from the host cell for successful reproduction and development. To better understand the intracellular parasite niche, we are using both time lapse live-cell imaging and a high-throughput EN technique called serial block-face scanning electron microscopy (SBF-SEM). This allows us to characterize both the dynamics of parasite interactions with host cell organelles as well as define the physical interactions between the parasite and the intracellular environment of the host. For *Encephalitozoon intestinalis*, one of the most common host tissues is the mucosal epithelial lining of the intestinal tract. It is composed of multiple cell lineages that are organized into a unique 3-dimensional crypt-villus structure, which is not well modeled by 2D cultured cell lines. Therefore, to begin to understand the parasite niche during replication in 3D tissues, we have used mouse intestinal organoids to create an *in vitro* cell culture infection model representative of the cell type diversity and complex architecture of the *in vivo* tissue niche. This will give us insights into how a human disease causing microsporidian parasite manipulates its hosts for successful growth and development.

**264D Activity of epigenetic inhibitors against Babesia divergens**

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Babesiosis, an emerging tick-borne parasitic disease of humans and livestock, has greatly increased in frequency and geographical range over the past few decades. Human infection can be severe in immunocompromised patients. Current treatment options are limited and treatment failure or intolerability are common. As epigenetic regulation in the human malaria parasite *Plasmodium falciparum* has offered promising targets for novel antimalarials, we decided to evaluate the activity of 324 epigenetic inhibitors against *Babesia divergens* blood stages. We identified 75 (23%) and 17 (5%) compounds that inhibited growth by over 90% at 10 µM and 1 µM, respectively. We observed differential activity of some inhibitor classes against *Babesia divergens* and *Plasmodium falciparum* parasites and compared the histone modifying enzymes orthologs between both species. Additionally, we identified pairs of compounds with high activity in activity, despite high similarity in chemical structure, highlighting new insights into the development of epigenetic inhibitors as anti-parasitic drugs.

**265D In vitro infection and genomic analysis of Crithidia-like parasites isolated from human cases of visceral leishmaniasis in Brazil**

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Leishmaniasis is a serious vector-borne infection transmitted by sandflies. Visceral Leishmaniasis (VL) is caused by protozoa from genus *Leishmania* and can be lethal when untreated or treatment fails. Results have shown that some clinical isolates obtained from an endemic region of VL in Brazil do not belong to *Leishmania* and are phylogenetically related to genus *monoxenous Crithidia* (i.e., they infect only one host – insects). Genome-wide mapping of *Crithidia*-like (LVH60a strain) Illumina reads to *Crithidia fasciculata* genome showed a nucleotide identity of 92%. Despite the high similarity between genomes, we suggest that they are likely different species. Because the monoxenous parasite *C. fasciculata* is considered non-pathogenic to humans, our aim was to identify the intracellular niche. This will give us insights into how a human disease causing microsporidian parasite manipulates its hosts for successful growth and development.

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**266D Environmental sensing and social motility are regulated by mechanosensation in Trypanosoma brucei**

Melvin Williams1, Tiffine Pham1, Monica Hernandez1, Kristy Nguyen1, Veronica Jimenez2 1) California State University Fullerton.

From the colonization of an insect vector to the infection of a mammalian host, *T. brucei* possesses a very dynamic and complex life cycle that encompasses a wide variety of environmental conditions. A mechanism by which these parasites can sense stimuli and regulate their intracellular conditions in response to these external changes must exist. In all organisms, mechanosensation is a key component of the pathways that allow for detection and response to environmental conditions. In bacteria, mechanosensation plays a role in pathogenesis by inducing the expression of virulence factors, regulating quorum sensing and biofilm formation.
formation. In protozoan parasites, the role of mechanosensation remains uncharacterized. We have identified a mechanosensitive ion channel (TbMscS) homologous to a small conductance mechanosensitive channel found in *E. coli*. TbMscS shares 64% identity with a recently characterized MscS-like channel in *T. cruzi* and 31% identity with *E. coli* MscS. Topology analysis reveals the channel has three transmembrane domains and conserved features in the pore forming region between TMD2 and TMD3. The protein is expressed in both life stages of the parasite, with slightly higher levels in procyclic forms. Endogenous tagging of TbMscS confirmed its localization to the mitochondria, as expected by its genetic homology with bacterial-like channels. Reduction of TbMscS expression by RNAi had no significant effect on the growth of the parasites, but it induced defects in cell volume regulation under hyper-osmotic conditions and abolished social motility behavior on semi-solid agar plates. Our results provide the first evidence of mechanosensitive-dependent functions in *T. brucei*, associated with environmental sensing.

267D The interface between the parasite *Trypanosoma brucei* and the mammalian host’s adaptive immune system Joey Verdi1, Johan Zeelen1, Alex Hempelmann1, Simone Weyand2, Nina Papavasiliou1, Erec Stebbins1 1) Deutsche Krebsforschungszentrum; 2) University of Cambridge.

The eukaryotic extracellular parasite *Trypanosoma brucei* causes long-term infections in mammals that rely on antigenic variation. Through this process, the parasite population is able to avoid immune-mediated elimination by periodically changing its surface coat. The surface coat is composed of approximately 10 million molecules of Variant Surface Glycoprotein (VSG) per cell. Trypanosomes genomes encode thousands of possible VSG alleles, although the genes are expressed monolaterally thus permitting the periodic changing of the coat through “VSG switching.” Here, we are beginning to use Cryo-electron microscopy (Cryo-EM) to visualize trypanosomal surface coats from cells expressing a variety of VSG variants with the intention of illuminating any differences in their three dimensional architectures. We will also attempt to visualize antibody bound surface coats by this technique in order to identify the predominant VSG epitopes that are recognized by the host’s humoral immune system during an infection, which currently remain uncharacterized.

268D Calmodulin-specific small interfering RNA induces consistent expression suppression and morphological changes in *Echinococcus granulosus* Seyed Mohammad mousavi1, Ali Afgar1, Mohammad Ali Mohammadi1, Seifollah Mortezaei1, Balal Sadeghi2, Majid fashi harandi1 1) Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman; 2) Shahid Bahonar University of Kerman, Faculty of Veterinary Medicine, Department of Food Hygiene and Public Health, Kerman, Iran.

Among parasitic helminths, biological features of Echinococcus granulosus have been a focus of particular interest in biology and medicine. The determinants and molecular mechanisms of Echinococcus development in different host settings is largely unknown. The phenominal bidirectional development of *E. granulosus* protoscolecites into multi-proglottid and/or microcysts, is a fascinating feature of the parasite cultivation. Calmodulin (CaM) is the major intracellular Ca2+ binding protein in plant and animal organisms. Many Ca2+-related processes in the physiology of eukaryotic organisms are CaM-dependent, however little is known on the role of CaM in platyhelminths growth and development. Small interfering (si) RNA-induced manipulations of the genes involving in the parasite development is an opportunity to explore novel approaches for cystic echinococcosis (CE) prevention and management. Regarding the fundamental role of CaM in cellular function of the parasites, in this study, we investigated the molecular and morphological changes induced by siRNA on CaM in different in vitro stages of *E. granulosus*. Three developmental stages of the tapeworm, protoscolecites, microcysts and strobilated worms, were cultivated in vitro in mono- and di-phasic media and three delivery methods, i.e. electroporation, soaking and electro-soaking, were used for RNA interference.

The level of mRNA suppression as well as the phenotypic changes of the parasites were measured. Following RNA interference, EgCaM mRNA suppressions of 65–99% were recorded in different stages of the tapeworm as compared to untreated/unrelated siRNA controls. Lower viability, growth retardation, morphological abnormalities as well as EgCaM expression suppression were documented in the parasite implying potential of siRNA technology for the prevention and management of CE.

269D Understanding Praziquantel Resistance in Juvenile Schistosomes at the Cellular Level Sarah Cobb1, George Wendt1, James J. Collins1 1) UT Southwestern Medical Center.

Schistosomiasis afflicts over 240 million people, killing hundreds of thousands every year and causing socioeconomic disease rivaling malaria. Schistosomes, the causative agent, are parasitic flatworms with a complex two-host life cycle. Adult worms reside in the vasculature of mammalian definitive hosts where they produce vast quantities of eggs, ultimately causing the pathology of schistosomiasis. Treatment relies upon a single drug, praziquantel (PZQ), that has significant drawbacks including a lack of efficacy against juvenile parasites. Indeed, the insensitivity of juvenile worms to PZQ is thought to be a major contributor to the limited efficacy of PZQ in the field.

It is unclear why juvenile parasites are refractory to PZQ. The drug causes tissue damage in both juvenile and adult animals and this damage can be at least partially repaired in *vitro*. One potential explanation for the juvenile’s hardiness comes from the observation that juvenile schistosomes have much higher numbers of somatic stem cells (termed “neoblasts”) compared to adult parasites. In adult schistosomes, these neoblasts are thought to be required for the parasite’s survival in the host by homeostatically maintaining tissues such as the skin and the gut, but their role in the juvenile is less clear. We hypothesize that the increased number of neoblasts in the juveniles allows them to repair PZQ-mediated damage better than adult parasites, leading to the lack of efficacy against juvenile animals.

In order to study the role of juvenile neoblasts in praziquantel resistance, we must first improve our understanding of the basic biology of the juvenile parasite. To this end, we are employing techniques such as single-cell RNAseq, in situ hybridization, and Edu-based fate mapping experiments to understand the molecular identity and function of neoblasts in this important stage of the schistosome lifecycle.

270D The Malaria Box compound MMV007113 targets the parasite Na+/H+ P-type ATPase, PHTP4 Suyash Bhatnagar1, Joanne M. Morissey1, Tarrick Qahash2, Manuel Lina2, Akhil B. Vaidya1 1) Center for Molecular Parasitology, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA; 2) Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, The Pennsylvania State University, Pennsylvania, USA.

We had previously screened the Malaria and Pathogen Box compounds to identify inhibitors of parasite lipid homeostasis (PMID: 30638365). We identified the compound MMV007113 that demonstrated lipid homeostasis disruptions independent of Na+ influx or inhibition of the sterol/H+ transporter, PINCH1. Published Na+ influx screens (PMID: 25145582) and meta-print reports (PMID: 27572391) on MMV007113 suggested that it might exert its action via the inhibition of a novel pathway regulating parasite plasma membrane lipid homeostasis. To discern the mechanism of action for MMV007113, two independent resistant parasites strains were generated. Sequencing of the resistant parasites revealed mutations in the Na+/H+ P-type ATPase PHTP4. Furthermore, real-time Na+ measurements also showed a rapid influx of Na+ into the parasite cytoplasm. We tested the cross-resistance of MMV007113-resistant parasite lines against other known PHTP4 inhibitors and observed a broad cross-resistance towards all inhibitors tested, except against the pyrazolamide PA220A92. Metabolomic assays comparing parasites treated with either MMV007113 or KAE609 revealed similar alterations consistent with both compounds inhibiting PHTP4. These results lend credence to the robustness of our lipid homeostasis disruption screen since MMV007113 was initially not seen to target PHTP4 or PINCH1 in the Na+ influx screen or metaprint analysis, but the lipid homeostatic disruption assay revealed its true mode of action.

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The flagellar attachment zone (FAZ) is a key morphogenetic structure regulating both cell length and organelle positioning in *Trypanosoma brucei*. Surprisingly, the *Leishmania* genome encodes homologs of most FAZ proteins albeit *Leishmania* lacking the laterally attached flagellum characteristic of *T. brucei*. In keeping with this finding, a discrete and short FAZ structure was recently identified in *Leishmania*; but the role of FAZ proteins in *Leishmania* remains enigmatic. Among the 34 genes encoding FAZ proteins, FAZ7 is the sole containing a kinesin motor domain, yet its function remains unknown. Kinesin FAZ7 orthologs are duplicated in the *Leishmania* genome. Here, we show that both *Leishmania* FAZ7 paralogs display different localizations and functions in L. mexicana. Kinesin FAZ7A localizes at the base of the flagellum, but the partial co-localization of the FAZ complex with a P. falciparum-based CRISPR-Cas9 approach, we generated FAZ7 single and double knock-out (FAZ7A+B) parasites. Null mutants of basal body-resident FAZ7A displayed normal growth rate and unmodified flagellar beat. By contrast, deletion of FAZ7B and double knock-out impaired cell growth in both promastigote and amastigote forms, albeit more drastically in the amastigote. The subcellular localization of FAZ and microtubule quartet components were essentially unmodified in FAZ7 KO cells. By contrast, the structure of the flagellar pocket collar (FPC) proteins BILBO1 and FPC4 appeared disorganized in these null mutants. They also grew significantly longer flagella and displayed cell division defects, with aberrant multinucleated and multi-flagellated cells showing abnormal nucleus and kinetoplast numbers. The ability of FAZ7B, but not FAZ7A, null mutants to proliferate in the sandfly vector and to induce lesions in mice was also impaired. Altogether, while FAZ7A appears like a basal body protein, dispensable for growth, in *Leishmania*, the kinetoplast FAZ7B is a protein of the FAZ complex, possibly involved in the transport of components essential to the biogenesis of the FPC, itself essential for cell growth.

### 272D Development of a CRISPR-Cas nuclease-based rapid diagnostic test for *P. falciparum* Holly Barkwill1, Heather Painter1 1) U.S. Food and Drug Administration, Center for Biologics & Evaluation Research, Division of Bacterial, Parasitic & Allergenic Products, Office of Vaccines Research & Review, Laboratory of Mucosal Pathogens and Cellular Immunology, Silver Spring, MD, USA.

Early diagnosis and treatment of malaria is imperative to reduce disease burden and transmission. Currently, there is no FDA-approved diagnostic test for malaria or standard platform for assessing the efficacy of anti-malarial drug products. Microscopy of thick blood smears and rapid diagnostic tests (RDTs) based on histidine rich protein-2 (HRP2) are the WHO recommended standards for detection of *Plasmodium* infection in human blood. However, both methods have disadvantages, as microscopy can be prone to false-negatives in low-level infections, and the emergence of *P. falciparum* HRP2 mutations threaten the efficacy of widely used RDTs. More recently, RT-PCR targeting *Plasmodium* 18S rRNA has offered a sensitive and specific alternative to traditional diagnostic platforms but have yet to be standardized for use in clinical trials and are not always viable options in resource-limiting settings. To improve upon existing malaria diagnostic detection platforms, we propose the use of CRISPR-Cas technology. CRISPR-Cas nucleases Cas12a and Cas13a have shown great promise for the development of next-generation molecular diagnostics technology for its ability to specifically target DNA and RNA, respectively, and their strong collateral and indiscriminate small molecule cleavage activity. These nucleases have been utilized in CRISPR diagnostic methods (PMIDs: 28408723 and 29449511) with greater sensitivity and specificity than traditional RT-PCR. Here we validate two CRISPR-based assays for the detection of *P. falciparum* 18S rRNA using Cas12a and Cas13a. By combining Recombinase Polymerase Amplification (RPA) with Cas-nuclease nucleic acid targeting in a “single-pot” assay that quantitatively measures the presence of 18S rRNA via a fluorescent reporter, we aim to detect this biomarker with greater sensitivity and more rapidly than previous diagnostic tests. Future work will determine the diagnostic sensitivity, specificity, and limit of detection of *Plasmodium* in human serum to verify the viability of this reaction in human plasma matrix. Optimization of these assays will enable rapid and sensitive detection of *Plasmodium* infections in clinical samples, which may be used for both evaluation of antimalarial drug and vaccine efficacy and as a novel point-of-care diagnostic in endemic countries, particularly where reported HRP2 mutations impede the use of existing RDTs.

### 273D A multi-omics approach to understand the mode of action of a Kalihinol analogue, a potent new antimalarial against *Plasmodium falciparum* Zeinab Chahine1, Jacques Prudhomme1, Maggie Daub1, Jonathan Chung3, Choukri Ben Mamoun2, Chris Vandewal3, Karine Le Roch1 1) Department of Molecular, Cell and Systems Biology, University of California, Riverside, United States.; 2) Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, Connecticut, United States.; 3) Department of Chemistry, University of California, Irvine, California, United States.

*Plasmodium falciparum* is responsible for the most severe form of human malaria. Its resistance to all current drug treatments threatens malaria control and elimination activities worldwide. Several Kalihinol natural products, members of the broader isocyanoterpene family (ICTs) of antimalarial agents, have shown to act as potent inhibitors against drug-sensitive and drug-resistant malaria parasites, suggesting that these compounds may target a novel metabolic pathway. Using complementary approaches including transcriptomics, proteomics and metabolomics, we explored the efficacy and mechanism of action of a Kalihinol analogue, MED6-189 on different stages of the human parasite life cycle of *P. falciparum*. Preliminary findings show a “delayed death” phenotype characteristic of a compound targeting the parasite apicoplast. These results were validated by the chemical rescue of the parasite organelle via isopentenyl pyrophosphate (IPP) treatment and the partial co-localization of the RFP-labeled molecules to the apicoplast. Affinity chromatography assay followed by mass spectrometry as well as an experimental approach that allows the identification of proteins stabilized upon ligand binding (CETSA) confirm a potential role in apicoplast function including vesicular trafficking. Gene expression and metabolomics profiling studies show no significant change in parasite development until parasite death in the second erythrocytic cycle consistent with our preliminary phenotypic analysis. Chemical in vitro evolution assay performed for more than 2 years selected parasites exhibiting a very small increase (2-fold) in the IC50 value. Whole genome sequencing of resistant clones is being performed to confirm the molecular pathway(s) affected by the drug. As a whole, our findings validate a complex mechanisms of action by which the parasite population collapses in the second cycle when treated with the drug. Understanding the exact mode of action of Kalihinol analogues on parasite population will allow structure-guided design approach to select not only more potent compounds but also to provide alternative strategies against potential drug-resistant parasites.

### 274D Different sensitivity to ruthenium-based inhibitors of the mitochondrial calcium uniporter complex of control and MICU1- and MICU2-ablated *Trypanosoma brucei* Mayara Bertolini1, Roberto Docampo1 1) University of Georgia.

The mitochondrial Ca2+ uptake in trypanosomatids shares biochemical characteristics with that of animals. However, the composition of the mitochondrial calcium uniporter complex (MCUC) in these parasites is quite peculiar, suggesting lineage-specific adaptations. In this work, we compared the inhibitory activity of ruthenium red (RuR) and Ru360, the most commonly used MCUC inhibitors, with that of the recently described inhibitor Ru265, on *Trypanosoma brucei*, and investigated whether MICU1 and MICU2 ablation increased the MCUC sensitivity to the inhibitors. The parasites were incubated with varying concentrations of MCUC inhibitors and we assessed the mitochondrial Ca2+ uptake by fluorescence changes of Calcium Green-5N in digitonin-permeabilized cells in the presence of succinate as substrate. When dose response curves were compiled, an increase in sensitivity for Ru360 was observed in MICU1-KO and MICU2-KO cells when compared with control cells. The half maximal inhibitory concentration (IC50) value for control cells was 134.3 ± 32.6 nM, while the values for MICU1-KO and MICU2-KO cells were 55.5 ± 22.7 nM and 74.7 ± 38.2 nM, respectively. In the presence of RuR, a significant increase in
sensitivity was observed only in MICU2-KO cells. The IC50 value for control and MICU1-KO parasites was 460 ± 29.8 and 379.7 ± 62.1 nM, respectively, while for MICU2-KO cells was 255.7 ± 102.6 nM. Dose-dependent inhibition studies showed that Ru265 was more potent than Ru360 and Ru8. When the IC50 values were calculated (26.8 ± 11.5 nM for control cells), no significant differences were found between the cell lines analyzed. The features that give Ru360 and Ru8 their potency and selectivity have not been fully elucidated. Our results suggest that the different ruthenium-based inhibitors do not have the same mechanism of action on T. cruzi MCUC.

275D Consequences of Na+ homeostasis disruption on the phosphoproteome of blood-stage Plasmodium falciparum Aarti Ramanathan1, David Sleat 3, Haiyan Zheng 3, Omar Harb 2, Akhil Vaidya1 1) Drexel University College of Medicine ; 2) University of Pennsylvania ; 3) Rutgers Medical School.

In the last decade, efforts to discover fast-acting and potent antimalarial drugs have led to the identification several distinct compounds that disrupt Na+ homeostasis in the malarial parasite. Na+ homeostasis disruptors target the Na+/H+ efflux pump, Plasmodium falciparum P-type ATPase 4 (PfATP4) and cause a rapid influx of Na+ into the parasite. Previously we reported that Na+ influx caused by PfATP4-targeting drugs induces an atypical schizont-like phenotype in a trophozoite stage parasite within 2 hours of drug treatment suggesting that Na+ influx might act as a signal for schizogony related events. To gain an understanding of molecular cues underlying these effects, we performed phosphoproteome of the parasites treated with two structurally distinct PfATP4-active drugs. Our results reveal a drastic reduction in phosphorylation of a large number of proteins after just 2 hour exposure to either pyrazolamide (100nM PA21A092) or spiroindoline (10nM KAE609), indicating that Na+ influx triggers large-scale rapid dephosphorylation of parasite proteins. We identified 385 proteins containing at least one significant phosphopeptide alteration after PfATP4-active drug treatment relative to the control. Functional enrichment analysis revealed alterations in proteins belonging to cell cycle progression such as DNA replication/repair, microtubule-organization, chromosome segregation and mRNA metabolism. Distinct among the alterations and of the highest significance was the glycolytic enzyme ATP-dependent phosphofructokinase (PFPK9), PFK9 was modified at two previously unreported positions, which were dephosphorylated after treatment with PfATP4 active drugs. Having previously shown that PfATP4-active drugs trigger premature schizogony, these data lead us to propose a metabolic-collapse to be a physiological requisite preceding schizogony.

276D Identifying specificity of histone reader domains in malaria parasites Jiacheng Liu1, Leen N. Vanheer2, Christopher Nōtzell1, Björn F.C. Kafsaacı2 1) BCMB Allied Program, Weill Cornell Medicine, New York, NY; 2) Department of Microbiology & Immunology, Weill Cornell Medicine, New York, NY.

Gene expression regulations, such as activation or repression, are largely driven by chromatin remodeling. The passage between different stages of Plasmodium life cycle requires extensive chromatin remodeling. Histone modification writers and erasers add or remove histone post-translational modifications (PTM), in order to change or maintain chromatin states. However, the recognition of these marks by proteins with histone reader domains is what gives these marks biological meaning by recruiting other modifiers, remodelers, or transcription complexes. Most of our current understanding of histone reader is derived from higher eukaryotes, with little discovered in the Plasmodium system. In order to understand the functions of histone reader domains, we started with identifying the histone PTMs that the readers bind to. As a proof-of-principle experiment, we have selected a subset of potential histone lysine methylation reader domains for analysis, including PHD, Chromo, and Zn-CW domains, with human and P. falciparum heterochromatin protein 1 (HP1) serving as positive controls. Candidate reader domains were expressed in E. coli and whole-folded reader domains were then assayed for binding against a panel of single modified nucleosome covering the possible methylation states of lysines residues on histones H3 & H4 using the dCypher assay based on AlphaScreen technology (Epicypher / Perkin-Elmer). This approach can be expanded to assay the specificity of a wide variety of reader domains in Plasmodium in order to decipher the histone code of malaria parasites.

277D Atypical Leishmaniasis in an endemic focus: Host and parasite determinants of disease outcome Nilokshi Samaranayake1, Sumudu Samarasinghe1, Hiruni Wijesooriya1, Hideo Imamur2, Nadira Karunaweera1 1) Faculty of Medicine, University of Colombo, Sri Lanka; 2) Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium.

Leishmaniasis constitutes several disease phenotypes ranging from self-healing cutaneous lesions to fatal visceral infections which are typically associated with different species of Leishmania. While interactions between the parasite, host and the vector are considered to influence the outcome, the determinants of these disease phenotypes are still poorly understood. Sri Lanka reports mostly localized cutaneous leishmaniasis (CL) with only a few cases of visceral disease diagnosed over the years. L. donovani MON37 is the only reported species in the country.

Clinical isolates from six cutaneous leishmaniasis patients (CL-SL) and two visceral leishmaniasis patients (VL-SL) were sequenced on an Illumina MiSeq platform to investigate parasite genomic variability. Peripheral blood mono nuclear cell (PBMC) derived macrophages and dendritic cells from newly diagnosed CL patients (n=60) and endemic (n=20) and non endemic (n=10) controls were stimulated with L. donovani antigen in vitro to assess the host immune response. The secretion of selected cytokines and NO were assayed at 24, 48 and 72hrs. The differences between experimental groups were analysed using Kruskal-Wallis test test for non-parametric data.

Chromosomal aneuploidy was observed in both groups but was more frequent in CL-SL. 248 genes differed by 2 fold or more in copy number among the two groups. Genes involved in amino acid use (LdBPK_271940) and energy metabolism (LdBPK_271950), predominated the VL-SL group with the same distribution pattern reflected in gene tandem arrays. Both chromosomal and SNP profiles showed CL-SL and VL-SL to form two distinct groups. The temporal cytokine profile showed a mixed inflammatory pattern with macrophages from patients producing higher levels of TNF, IL10, TGF β and nitric oxide. Dendritic cells from patients produced higher levels IL12 and IL10 (p<0.05).

Comparison of parasite isolates suggests that intra species variations at chromosome and gene level are more likely to influence differences in tropism. The host cytokine responses should be interpreted in the context of changes in other inflammatory mediators and compared to immune responses of patients with visceral Leishmaniasis caused by the local parasite to better understand the underlying pathogenic mechanisms.

278D The first evidence of operon gene clusters in Orthonectidae genomes DARIA SHAFRANSKAYA1, Natalya Bondarenko1 1) Saint Petersburg State University.

Orthonectidae is a small, rare, and in many aspects enigmatic group of organisms with a unique life cycle and a highly simplified adult free-living stage parasitizing various marine invertebrates. Several studies have shown that parasitism can lead to a dramatic reduction of the body plan, morphological structures and also affects organisms at the genomic level. Several studies were shown that Orthonectidae has extremely compact genomes due to the significant reduction of gene number, intergenic regions, intron length, and repetitive elements. As a result, gene density in these species is much higher than in other parastomes. The close proximity of Orthonectidae genes suggested that they might possess operon organization and co-transcribed from a single upstream promoter. In this study we sequenced I. linei transcriptome and combined the experimentally determined polycistronic transcripts with computational analysis of intergenic distances in the I. linei genome. The most recent genome assembly for the Orthonectidae Intiosha linei has 51.5% of genes that might be organized into operons. Whereas transcriptomic data confirmed 11.25% of the predicted genes which organized into polycistronic operons. Orthonectid genes are transcribed without the involvement of trans-splicing processes and translated as a classical prokaryotic operon. Most of the annotated polycistronic transcripts are functionally related to proteins responsible for the processing of genetic information. This is the first and unique example of the organization of transcription of the eukaryotic genome by prokaryotic type on such a significant scale.

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In Trypanosoma cruzi, the etiological agent of Chagas’s disease, amino acids participate of several critical processes in the parasite biology, such as osmoregulation, cell differentiation and host cell invasion. Some of them provide reducing power for mitochondrial ATP synthesis. We recently demonstrated that L-Ala, can be a metabolic source of glycolysis, and can be a nutrient which can be taken up to fuel the oxidative phosphorylation. In this work we show that T. cruzi encodes a functional Alanine Racemase (TcAR). The enzyme was cloned and expressed and its recombinant version (rTcAR), and was biochemically characterized. Km and Vmax were determined for the reaction in both directions (21.5 ± 6.04 mM and 65 ± 8.7 µmol/min/mg (D to L) and 21.1 ± 7.9 mM and 53 ± 6.3 µmol/min/mg (L to D)). Additionally, we showed that D-Ala can be taken up by epimastigotes through a low specificity non-stereoselective active transport system. D-Ala uptake happens with a Vmax of 1.14 ± 0.75 nmol/min·1 per 2 x 107 and a Km of 1.46 ± 1.4 mM. The incorporated D-Ala can be completely oxidized to CO2, supplying electrons for the oxidative phosphorylation. Of note, D-Ala is not transaminated by the parasite transaminases. These data suggest that the D-Ala oxidation mainly occurs through the TcAR. To verify this hypothesis, we generated a lineage of T. cruzi Brno hemi-knockout for TcAR (TcAR+) using CRISPR-Cas9 technology, and we characterised the phenotype of the TcAR+/- parasites. TcAR+/- epimastigotes, when compared to Cas9-expressing controls, showed a decrease in their proliferation rate and an increased sensitivity to nutritional stress. As expected, TcAR+/- epimastigotes showed a diminished viability in presence of D-Ala, when compared to controls. Additionally, TcAR+/- epimastigotes incubated with [14C(U)]-D-Ala, exhibited a decrease of the radiolabelled CO2 compared to the control cell lines, indicating the successful impairment of the D-Ala degradation pathway. Our results point that TcAR is the key enzyme for D-Ala oxidation and appears to be involved in other biological process such as resistance to nutritional stress and parasite replication. To conclude, the metabolism of DL-Ala and the related AR activity underlines the outstanding metabolic flexibility and the relevance of the D-amino acids metabolism in these organisms.

280D Characterizing RNA-binding proteins essential for Plasmodium falciparum sexual development Andrew Stasic1, Heather Painter1 1) US Food and Drug Administration, Silver Spring, MD.

Plasmodium falciparum malarial disease remains a significant global health burden that results in hundreds of thousands of deaths annually with current treatments becoming increasingly less effective. Efforts to eradicate P. falciparum will likely rely on the disruption of sexual development as this stage is critical to the transmission of parasites and the persistence of malaria within the human population. Understanding gene regulatory mechanisms and factors that enable maturation of the transmissible sexual stages is essential to providing novel methods for eradication. Studies that genetically ablate or disrupt the expression of critical gametocyte-specific RNA binding proteins (RBPs) indicate the complex networks of post-transcriptional regulation and translational repression required during sexual development. However, the exact roles of essential sexual-stage RBPs remain poorly characterized in human malaria. In this study, we aim to identify the in vivo mRNA targets of five RBPs important in Plasmodium sexual development that includes PUf2, DOZi, CITH, Pf27, and ALB2A. These RBPs have been previously demonstrated to be important for sex-designation, gametocyte maturation, and oocyst formation/maturation in Plasmodium. To identify the target RNAs of each RBP, we first transfected P. falciparum strain NF54 parasites with two selection-linked GFP-tagging integration plasmids with differing functions: one to create a permanent knockout and another to create a GFP-tagged conditional knock-down/side mutant. Additionally, we could use the yeast fusion of uracil phosphoribosyltransferase (yFCU) to enable biosynthetic labeling of RNA and consequent photo-crosslinking to any zero-distance RBP-RNA interactions. Following immunoprecipitation of crosslinked RBP-RNA complexes, novel sequencing techniques will identify the RNAs bound by each RBP as well as the RNA-interaction motif. These data will contribute to our understanding of the crucial gene regulatory networks involved in sexual development and reveal the molecular post-transcriptional regulatory events required for gametocytogenesis.

281D TgOTUD5, an essential phosphorylation-regulated deubiquitinase, controls Toxoplasma gondii plasma membrane homeostasis Animesh Dhar1, Robert Murphy2, Anthony Sinai1 1) University of Kentucky College of Medicine, Department of Microbiology Immunology and Molecular Genetics ; 2) University of Kentucky College of Medicine, Department of Molecular and Cellular Biochemistry.

Post translational modifications, including ubiquitination and phosphorylation control the fidelity of the Toxoplasma cell cycle. Here we examine the contribution of an essential cell cycle regulated deubiquitinase TgOTUD5 (TGTG1_243510). Immunofluorescence analysis shows TgOTUD5 is cytoplasmic and exhibits dynamic expression and localization across the cell cycle. TgOTUD5 expressed in E.coli exhibited modest activity against polyUb (K63→K48→K11-linear poly-Ub synthetic substrates). Notably, TgOTUD5 expressed in insect cells (sF9) which are capable of phosphorylation, exhibited significantly enhanced activity. Activity was reduced upon phosphate treatment, indicating phosphorylation mediated regulation of TgOTUD5 activity. Both targeted disruption of TgOTUD5 and the conditional KO using the Auxin-AID system proved unrecoverable. Success was achieved with the N-terminal HA-tagged Tet-off system which, under knock-down conditions (+ATc), resulted in a marked growth defect and invasion defect. Prominent underlying causes behind these defects appear to be associated with accumulation of SAG1 in intracellular vesicles, release of SAG1 in the PV, numerous other structural abnormalities of the plasma membrane (empty PM vesicles in the PV, blebs (discontinuity with the IMC), dendrite-like projections), and aberrant IMC formation. These data suggest that TgOTUD5 plays a role in the coordination of plasma membrane homeostasis likely by affecting its trafficking and sorting, consistent with its preference for the K63 Ub linkage. The role of TgOTUD5 phosphorylation in vivo was confirmed by detection of mono-phosphorylation events at the C-terminus, with S375-P being the most prominent. We were unable to recover a TgOTUD5(S375A) line. We therefore introduced this allele as a second copy in the regulatable Tet-off TgOTUD5 line. The presence of the mutant allele resulted in a growth defect and phenotypic changes similar to the knock-down despite the presence of the WT protein indicating a strong dominant negative effect. This was reinforced by the fact that the selective ablation of the WT protein did not alter the overall effect on growth and associated phenotypes. Taken together, TgOTUD5 is an effector impacting Ub-mediated processes linked to membrane traffic that is controlled by phosphorylation, thereby highlighting a sophisticated system to establish and maintain plasma membrane homeostasis along with the fidelity of cytokinesis.

282D Unabated malaria transmission in a peri-urban community of Akure, southwestern Nigeria: A cross-sectional and Hospital-based study of the prevalence, intensity and risk factors enhancing transmission Oluwaseun Awoosolu1, Kehinde Elegbede1, Titus Olus1 1) Federal University of Technology, Akure, Nigeria.

Malaria is a vector borne infectious disease caused by Plasmodium spp. It is a serious public health problem worldwide particularly in sub-Saharan Africa. This study was conducted to investigate the prevalence, intensity and risk factors affecting malaria transmission in a peri-urban community of Akure, southwestern Nigeria. Thick and thin smear were prepared and examined under ×100 objective lens of the light microscope to ascertain the prevalence of Plasmodium falciparum in blood samples. Questionnaires were employed to collect information such as sex, age, location and variables for economic status. Out of 300 subjects who volunteered, 235 (78.3%) were infected with P. falciparum. Females had a lower infection prevalence of 125 (76.7%) compared to their male counterparts with 110 (80.3). Age group is related to the malaria infection in the study area. While the highest rate of infection was observed among ≤5 years, the least was observed among the subjects within the age group 11-15 years (P<0.05). Similarly, with parasite density, males had lower parasite density (180 parasite/µL of blood) compared to female who have a higher parasite density (2068 parasite/µL of blood). In the same vein, those who are more than 21 years had lower density as against those of ≤5 years. Economic variable which is determined by income was significantly related to malaria infection (P<0.05). Subjects earning ≤18000 Naira/month had higher malaria prevalence of 85.2% while subjects earning ≥31000 Naira/month had lower malaria prevalence (56.9%). There was
significant difference (P<0.05). The outcome of this current study is a proof that malaria is prevalent in the study area and appropriate control intervention should be made available to the populace to ameliorate infection condition.

283D Nucleosome landscape differs at strategically genomic regions in replicative and non-replicative forms of Trypanosoma cruzi

Trichomonas vaginalis alternates between replicative and non-replicative forms accompanied by a shift on global transcription levels and by changes in its chromatin proteome, post-translational modifications (PTMs) and architecture. To gain insights about epigenetics regulation that follow life forms differences, we performed a genome-wide high-resolution nucleosome mapping using replicative and non-replicative T. cruzi life forms. By combining a powerful pipeline that allowed us to faithfully compare among nucleosomes landscape of different life forms, more than 125 thousand nucleosomes were mapped and about 20% of them are considered different among life forms either (or both) at their occupancy level, fuzziness state or genomic location. Remarkably, T. cruzi nucleosome landscape differs at strategically genomic regions. Regions associated to transcription start sites (TSS) are more affected by the nucleosome dynamism than all other genomic features and is mainly more enriched in nucleosomes at trypomastigote forms. Comparing intragenic regions, multi-family genes – mainly associated with infective-stage and virulence factors have also more dynamic nucleosomes, highlighting the DGF-1 genes, which 80% of its members contain at least one dynamic nucleosome. Finally, the nucleosome landscape reflects steady-state transcription expression: more abundant genes have a deeper nucleosome-depleted region at a putative 5’ splice site. Taken together, our results pointed out that chromatin architecture, held primarily by nucleosome positioning, reflects some phenotypic differences found at T. cruzi life forms.

284D Dissecting primary human erythroblast responses to Plasmodium falciparum infection

Malaria anemia is a large and understudied contributor to maternal and infant mortality in areas of endemic malaria. Severe anemia results from the combination of red blood cell (RBC) destruction and aberrant production (dyserythropoiesis). While the pathogenesis of dyserythropoiesis in malaria is not well understood, both asexual and sexual stage Plasmodium falciparum parasites have been observed in the bone marrow in clinical samples, suggesting they may impact erythroid development, either directly or indirectly. In vitro studies have demonstrated that P. falciparum is capable of infecting late-stage erythroblasts and that incubation of parasites or parasite metabolites with erythroid progenitor cells can perturb host cell proliferation and induce transcriptional changes in bulk culture. However, the host cell responses to infection remain unknown, as do the specific changes that drive dyserythropoiesis.

To characterize the host cell responses to P. falciparum infection of the hematopoietic niche, we designed and implemented an approach to infect primary human erythroid progenitors cells at discrete stages of differentiation and measure the stage-specific transcriptional responses of infected versus exposed cells by RNA-seq. We induced primary CD34+ hematopoietic/stem progenitor cells to proliferate and differentiate down the erythroid lineage and inoculated them with late-stage, GFP-expressing P. falciparum on day 7, 10, or 14. Using an MOI of 5, we observed robust infection of erythroid progenitor cells at all stages of terminal differentiation and demonstrated that parasites were viable until at least 20 hours post-invasion. In a pilot RNA-seq study to profile the stage-specific host transcriptional responses to P. falciparum, we sorted erythroblasts at specific stages of differentiation and sequenced mRNA from unexposed, exposed, and infected cells ~20 hours post-invasion. We identified more than 150 differentially expressed genes in infected versus exposed or unexposed cells at each differentiation stage. Gene set enrichment analysis revealed alterations in expression of cell cycle pathways, immunologic responses, and apoptosis in infected versus exposed cells. We also detected parasite transcripts in infected erythroblasts that are hallmarks of ring-stage development.

In ongoing work, we are extending our transcriptomic study to include all stages of terminally differentiating erythroblasts to determine how developmental stage impacts host cell responses to P. falciparum and assessing how parasite effectors may impact the observed host cell responses. This information will inform functional studies aimed at dissecting the molecular interactions between host and parasite that contribute to dyserythropoiesis.

285D Phenotypic Characterization of an Essential Myzozoan-specific Mitochondrial Protein with Unknown Function in Plasmodium falciparum

Mitochondrial functions are essential throughout the life cycle of malaria parasites and have been validated as targets of antimalarial drugs. We are exploring the possibility that essential mitochondrial functions may provide additional targets for the discovery of novel antimalarial drugs. Here, we seek to characterize a Plasmodium protein encoded by the gene PF3D7_0105500 with unknown function. This gene is highly conserved and limited only to the Myzozoan superphylum. Using a conditional gene knockdown, we derived a transgenic parasite line in which the PF3D7_0105500 gene product is endogenously tagged in a manner that expression is dependent on the presence of anhydrotetracycline (aTc). Withdrawal of aTc caused parasite demise, showing essential function of this gene for parasite survival. We have successfully expressed this protein recombinantly in E. coli to direct antibody production as well as to assess its potential function. We also generated a parasite line in which this gene is overexpressed from an ectopic site and tagged with 3HA, which allowed us to detect the protein by Western blotting and to localize it to the parasite mitochondrion by immunofluorescence. Lastly, we conducted experiments to look for hypersensitivity to antimalarials with known mitochondrial targets after PF3D7_0105500 knockdown including atovaquone, proguanil, and DSM1 as judged by titrated hypoxanthine.

286E Stage-specific downregulation of SPATR impacts malaria sporozoite motility and infectivity

Malaria, a vector-borne disease caused by Plasmodium species, remains one of the most concerning public health problems, having caused an estimated 228 million cases and 405,000 deaths in 2017. The clinically silent pre-erythocytic phase of infection has long been established as an attractive target for the development of a much-needed protective malaria vaccine. We therefore made use of reverse genetics and in vivo bioluminescence imaging to study the involvement of several sporozoite molecules in this process. One of these candidate proteins is the Secreted Protein with an Altered Thrombospondin Repeat (SPATR), which contains adhesive domains and is highly expressed in mature sporozoites, suggesting a function in the early stages of infection. Conditional spatr knockouts in sporozoites demonstrated SPATR is required for blood stages but no defect was observed, either during the mosquito or the pre-erythrocytic phases2. However, incomplete excision of the gene and transcription during early oocyst development associated with translational repression3 could explain
unimpaired progression through these phases of the life cycle. Thus, we alternatively devised a promoter swap strategy that would achieve knockdown of \textit{spatr} in oocysts. Our results indicate that invasion of the mosquito salivary glands by knockdown (KD) sporozoites is impaired. Moreover, KD sporozoites collected from the hemolymph of the vector and injected intravenously in mice could not produce detectable liver loads and subsequent blood infections. KD salivary gland sporozoites also showed impaired infectivity \textit{in vivo}. This infectivity defect is further supported by lower numbers of KD parasites entering and developing inside a hepatoma cell line in comparison to the control lines. The motility of both hemolymph and salivary gland sporozoites \textit{in vitro} is also compromised. In conclusion, SPATR emerges as a potential target to be considered in vaccination strategies due to its involvement in several stages of the \textit{Plasmodium} life cycle.

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287E Discovering new targets for a malaria vaccine Ana Rafaela Teixeira1,2, Begoña Perez-Cabezasi,2, Mónica Sai,2, David M. Costa1,2, Sylvain Golba3, Rogerio Aminoi, Anabela Cordeiro-da-Silva1,2,5, Joana Tavares1,2 1) I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; 2) Parasite Disease Group, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; 3) Center for Production and Infection of Anopheles, Institut Pasteur, Paris, France; 4) Unit of Malaria Infection and Immunity, Institut Pasteur, Paris, France; 5) Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

Malaria is the deadliest parasitic disease in the world and despite decades of research, a highly effective human vaccine against its etiological agent, \textit{Plasmodium}, remains elusive. In the search for antigens for a malaria vaccine we have devised a novel strategy to identify on the surface of the sporozoite - the parasite stage inoculated in the skin by a mosquito bite - potential targets of infection-blocking antibodies, to be explored as vaccine candidates. For this purpose, we generated specific immune repertoires in mice following intradermal immunizations with irradiated sporozoites, the gold standard in malaria vaccines. These repertoires were isolated in the form of their Fragment of antigen binding (Fab) sequences and then combined with antibody phage display technology to create Fab libraries. Two libraries of 94 Fab each were screened for their capacity to bind the sporozoites' surface and their non-reactivity to the sporozoites' most abundant surface protein, the circumsporozoite protein (CSP). We identified a total of 111 Fab that fit these conditions, and which were sequenced. Of these, 43% (53 Fab) had valid sequences, of which 7 shared the same complete sequence as a CSP-specific Fab. Besides the overall sequence, the complementary determining region 3 (CDR3) of the Fab is also important as the major determinant of its specificity. Analysis of the sequences revealed 11 Fab with unique CDR3 sequences. The 46 Fab whose overall full sequence was different from CSP-specific Fab, were tested in an immunofluorescence assay on sera-activated sporozoites. This assay revealed distinct patterns of binding for several of the Fab, suggesting they might be binding different proteins on the surface of the sporozoite. Our next step is the identification of these proteins and evaluating whether active immunizations can generate antibodies capable of blocking parasite infectivity.

Thus, we expect that this approach, will aid in the discovery of immunogens to use in vaccination strategies and that can potentially drive the production of high affinity antibodies capable of blocking sporozoite infectivity.

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The frontline antimalarial drug class artemisinin (ART) has been critical to malaria control. However, countries in Southeast Asia, South America, and most recently, Africa have reported clinical failures of ART and artemisinin combination therapies, threatening the efficacy of this drug. Previously, we investigated the genetic determinants of ART resistance in \textit{Plasmodium falciparum} strains from Africa, where the highest burden of malaria is found. Using Senegalese isolates from Pikine and Thies, we performed \textit{in vitro} evolution to generate two independent ART-resistant \textit{P. falciparum} parasite lines. We identified mutations in the actin-bundling protein, \textit{PfCoronin}, in both lines. To evaluate the gain- and loss-of-function in resistance associated with mutations in \textit{pfcoronin}, we generated CRISPR-Cas9 edited parasites and found that \textit{PfCoronin} is sufficient to confer ART resistance, as measured by the ring-stage survival assay (RSA). Emerging ART resistance has also been attributed to mutations in \textit{PFKelch13}, which was recently found to disrupt endocytosis in mutants. Considering that \textit{PfCoronin} has actin-binding activity and the \textit{Toxoplasma gondii} homolog is involved in vesicular trafficking, we examined the potential resistance synergy between \textit{PFKelch13} and \textit{PfCoronin}. Interestingly, both proteins also have a WD-40-beta-propeller domain. We generated single and double mutant parasites with \textit{pfcoronin} (R100K & E107V) and \textit{pfkelch13} (C580Y) mutations in the Pikine and 3D7 genetic backgrounds. This study is the first report of \textit{pfkelch13} mutants in the Pikine background, which had significantly higher RSA survival in two clones (28% and 42%) relative to the wildtype (RSA<1%). ART resistance (measured by RSA) in parasites with mutations in both genes was similar to that observed in \textit{PFKelch13} single mutants, suggesting that the resistance contribution of \textit{pfcoronin} is masked by that of \textit{pfkelch13}. Two clones of double mutants in the Pikine background had an RSA survival of 27% and 33%, while the \textit{pfcoronin} mutant in the same background had a survival of 9%. This phenomenon was also observed in the 3D7 background, implying that \textit{pfcoronin}-mediated ART resistance may occur in the same pathway as \textit{pfkelch13}. Future studies on \textit{PfCoronin} biology as well as interactions with other proteins in the Senegalese parasite lines can elucidate unique pathways exploited by ART-resistant parasites in the field.

289E The Architecture of the Native Apicomplexan Nuclear Pore Complex Pravin Dewanog1, Kai Cai2, William O'Shaughnessy1, Xiaoyu Hu1, Daniela Nicastro2, Michael Reese1, 3) Department of Pharmacology, UT Southwestern Medical Center, Dallas, Texas 75390.; 2) Department of Cell Biology, UT Southwestern Medical Center, Dallas, Texas 75390.; 3) Department of Biochemistry, UT Southwestern Medical Center, Dallas, Texas 75390.
The nuclear pore complex (NPC) is a multisubunit proteinaceous assembly embedded in the nuclear envelope and it regulates the exchange of macromolecules between the cytoplasm and the nucleoplasm. Although the general architecture of NPC is conserved among diverse eukaryotes, there are differences in its components and their organization. The mammalian NPCs are larger in size with certain subcomplexes present in multiple copies to support specific structures like the cytoplasmic filaments that are absent in yeast. There are several nucleoporins (Nups) that are mutually exclusive between different species. In apicomplexan parasites like *Toxoplasma gondii*, a handful of Nups have been identified based on sequence conservation and their localization have been confirmed at the nuclear envelope. However, several critical components of nuclear transport remain elusive, including the RanGAP and mRNA export platform. In this study, we aim to determine the molecular structure and interactome of the apicomplexan NPC. Using cryo-electron tomography (cryo-ET) and focused ion-beam milling on flash-frozen parasites we are determining the structure of the apicomplexan NPC in its native functional state. The cryo-ET structure is crucial for visualizing its architectural details and comparing it to NPC structures from other species; thereby identifying unique features of the apicomplexan NPC.

To identify proteins that are specific to the apicomplexan system we are using BioID to compare the interactomes of Nups localized at the central channel, the adapter region and the linker to Y-shaped complex in the NPC. Overall, with this study we are highlighting the unique proteins and features of native functional apicomplexan NPC.

290E Plasmodium SAXO1 is a conserved, redox-active protein that binds and stabilizes microtubules Stefan M Kanzok1, Griffin Berge1, Farah Siddiqi1, Filza Ali1, Chase Gauthier1, Kaitlyn Kiernan2 1) Loyola University Chicago, Lake Shore Campus, Chicago, IL; 2) University of Illinois Chicago, Chicago, IL.

Microtubules are highly dynamic structures that play vital roles in the function and development of eukaryotes, including protozoan parasites. The assembly, disassembly, and stability of microtubules are delicately controlled by microtubule-associated proteins (MAPs) only very few of which have been characterized in the malaria parasite *Plasmodium*. Here we describe PbSAXO, a MAP6-related protein, in the rodent malaria parasite *Plasmodium berghei*. Homologous proteins have been recently described in *Toxoplasma* and *Trypanosoma*, respectively. PbSAXO is an unstructured, 38 KDA protein that consists of a cysteine-rich head domain, followed by five nearly identical 32 amino acid repeats, and a short conserved carboxy-tail. Recombinantly expressed and purified PbSAXO is readily reduced by the P. berghei thioredoxin system in vitro exhibiting saturation kinetics. Using a heterologous expression system we show that PbSAXO binds to and cold stabilizes microtubules. The deletion of the gene, using CRISPR Cas9, shows that PbSAXO is dispensable for the asexual development of *P. berghei* in mouse blood. Investigations are underway to confirm the microtubule-binding activity of PbSAXO in parasites and to determine its potential function in the development and survival of *Plasmodium* ookinetes and sporozoites.

291E The rhythmic day of malaria parasites Filipa Rijo-Ferreira1,2, Victoria Acosta Rodriguez1, John H Abel1, Ines Bento4, Maria M Mota4, Joseph Takahashi1,2 1) UT Southwestern, Dallas, TX; 2) Howard Hughes Medical Institute; 3) Massachusetts Institute of Technology, Boston, MA; 4) Instituto Medicina Molecular, Lisbon, Portugal.

Our rhythmic world has been a driving force for organisms to evolve a clock to anticipate such rhythms. Similarly, our own circadian biology leads to body rhythms that parasites experience. Malarial rhythmic fevers are the consequence of the synchronous bursting of red blood cells (RBCs) on completion of the malaria parasite asexual cell cycle. How is this bursting synchronous across the parasite population? Are parasites following host cues or do they also have a clock to anticipate host daily rhythms? Through a combination of infection challenges where we manipulate the environment or rhythms of the host by infections of circadian mutant hosts we propose malaria parasites to have intrinsic clocks. Furthermore, parasite stages that have been considered quiescent in the mosquito appear to have daily rhythms that impact infection efficiency. Thus, parasite rhythms are aligned to the host daily rhythms but are generated by the parasite, possibly to anticipate its circadian environment.

292E Demonstration of genetic exchange between New World and Old World strains of Leishmania with different karyotypes Eliza Vanessa Carneiro Alves Ferreira1, Ehud Inbar1, Tiago Rodrigues Ferreira1, Justin Lack1, Asis Khan1, David L. Sack1, Michael E. Grigg1 1) National Institutes of Health.

The natural occurrence of cross-species hybrids of Leishmania are well described. Given our success of recovering experimental hybrids from sandflies co-infected with different strains of *Leishmania*, we sought to test whether *Leishmania* species with different karyotypes are mating compatible and produce fertile progeny. Using *Lutzomyia longipalpis*, a sandfly species that is permissive to both New World (*L. amazonensis*; 34 chromosomes) and Old World (*L. major*; 36 chromosomes) species of *Leishmania*, we successfully generated thirteen independent F1 hybrids and five independent F2 backcross (BC) hybrids, each isolated from a different sandfly. The F1 hybrids were heterozygous at all homozygous SNP markers that differentiate the two parents, indicating that they were full genomic hybrids. Progeny showed a mixture of karyotype profiles and DNA content, according to whole-genome sequencing (WGS), pulse field gel electrophoresis (PFGE) and flow cytometry analyses. Consistent with previous crosses, different signatures of aneuploidy, CNV, gene dosage, and loss of heterozygosity were observed. Importantly, our results formally demonstrate that F1 progeny from these inter-species crosses are fertile. Analysis of the F2 backcross progeny identified distinct but limited cross-over events genome-wide, consistent with sexual recombination. Clear phenotypic differences for a variety of traits (for example, vacuole size) exist between *L. amazonensis* and *L. major*, and we are currently performing additional backcrosses to generate more progeny to map genes associated with vacuolar Quantitative Trait Locus (QTL) and Whole Genome Wide Association Studies (GWAS).

293E Exploring the uncertainty of protein localization in Toxoplasma by Bayesian analysis of the spatial proteome Konstantin Barylyuk1,2, Oliver Crook2,3, Kathryn Lilley2, Ross Waller1 1) Department of Biochemistry, University of Cambridge, Cambridge, UK; 2) Cambridge Centre for Proteomics, Department of Biochemistry, University of Cambridge, Cambridge, UK; 3) MRC Biostatistics Unit, Cambridge Institute for Public Health, University of Cambridge, Cambridge, UK.

One of the key mechanisms of regulatory control in the cell is compartmentalization. It establishes a specific molecular environment where gene products – in particular, proteins – fulfill their function. Apicomplexan cells are a vivid illustration of this principle: they include both generic eukaryotic organelles, and specialized subcellular compartments and structures with roles in parasites’ interactions with hosts, survival, propagation and transmission. The functional profiles of the subcellular niches are defined by their proteomes that are a product of evolutionary adaptation and innovation, as well as reduction.

Recently, we applied the spatial proteomics technology Hyperplexed Localization of Organelle Proteins by Isotope Tagging (hyperLOPIT) to a model apicomplexan parasite *Toxoplasma gondii*. By measuring protein co-fractionation upon mechanical disruption and biophysical fractionation of extracellular tachyzoites, hyperLOPIT simultaneously assigned 3,832 Toxoplasma proteins to 26 distinct subcellular niches. This enabled a dramatic expansion of the known compartment proteomes and provided valuable insight into the biochemical, functional, regulatory, and evolutionary landscape of the apicomplexan cell.

HyperLOPIT provides a steady-state snapshot of the spatial proteome, however, many proteins in the cell reside in, or dynamically redistribute between multiple locations. Such proteins feature composite abundance distribution profiles and cannot be unambiguously attributed to a single subcellular niche. In *Toxoplasma*, approximately a third of the proteins captured by hyperLOPIT could not be robustly assigned to a defined location. Here we apply a Bayesian framework to the analysis of *T. gondii* spatial proteomics data to quantify the uncertainty of protein location prediction by the t-augmented Gaussian mixture model with Markov-Chain Monte-Carlo inference of the model parameters (TAGM-MCMC). We identify the cohorts of proteins with a large degree of uncertainty in location inference. Importantly, the method distinguishes when this uncertainty is simply due to noise in the data. We interrogate the posterior protein localisation probability distributions to interpret the observed uncertainty, which in many cases points to multi-localized proteins. Our approach provides further insight into the spatial distribution of *Toxoplasma* proteome and reveals subcellular hotspots of protein location dynamics.
294E Genome-wide CRISPR/cas9 screen identifies host factors essential for optimal Plasmodium liver stage development. Kamalakannan Vijayan1, Nadia Arang2, Ling Wei1, Robert Morrisson1, Rechel Geiger3, Rachael Parks4,5, Adam Lewis1, Fred Mast1, Alyse Douglass4, Heather Kain6, John Aitchison1, Jarrod Johnson7, Alan Adere1, Alexis Kaushansky1,4, 8, 9 1 Seattle Children’s Research Institute; 2 University of California, San Diego ; 3 MSTP program, University of Washington, Seattle, Washington; 4 Department of Global Health, University of Washington, Seattle, Washington; 5 Fred Hutchinson Cancer Research Center, Seattle, Washington; 6 Bristol Myers Squibb, Seattle, Washington; 7 University of Utah, Salt Lake City, Utah; 8 Brotman Baty Institute, Seattle, Washington, Seattle, Washington; 9 Department of Pediatrics, University of Washington.

Prior to initiating symptomatic malaria, Plasmodium parasites infect and develop within hepatocytes. We performed a forward genetic, genome wide CRISPR/Cas9 screen to identify host regulators of Plasmodium liver infection. Single guide RNAs involved in vesicle trafficking, cytoskeleton organization and lipid biogenesis altered Plasmodium liver stage development. We observed a close association between Golgi derived vesicles and fragmented Golgi stacks with the parasite periphery. The host microtubule network and non-centrosomal microtubule organizing centers closely associated with the parasite; we predict to facilitate vesicle trafficking. Knocking out the centrosomal MTOC protein CENPJ further exasperated the localization of MTOCs to the parasite and increased infection, suggesting that the parasite can either utilize nMTOCs or assemble them using non-canonical factors. In addition to elucidating a mechanism by which parasites sequester host material, our data provide a wealth of yet untested hypotheses about the elusive biology of the liver stage parasite.

295E Investigating nonenzymatic functions of an essential citrate synthase-like protein in Plasmodium falciparum Sezin Nicklas1, Michael Mathler1, Hangjun Ke1, Akhil Vaidya1 1 Drexel University College of Medicine.

We have previously demonstrated the dispensability of six of the eight TCA cycle enzymes for normal asexual blood stage metabolism and growth in P. falciparum (Ke et al. 2015. PMID:25843709.) Of these enzymes, the viability of a citrate synthase (CS) knockout transgenic parasite line could have been due to a functional redundancy from a putative citrate synthase-like protein (CSL, P1507_0069200) found in the genome. However, despite belonging to the CS superfamily, CSL does not contain the conserved catalytic residues within the signature domains of CS. This suggests that CSL cannot perform CS function in vivo. We then successfully germinated a transgenic parasite line in which the endogenous CSL locus was conditionally expressed under the TetR-DO2I regulation. The protein is detectable at the correct size, and knockdown was verified by Western blot. Upon knockdown of CSL, parasites ceased to grow within 48 hours. It is noteworthy that the citrate synthase (CIT1) of the ciliate T. thermophila polymerizes into filaments. As a structural protein in the cytoplasm, CIT1 is involved in formation of T. thermophila’s oral apparatus, a structure with similarity to the cytostomes of malaria parasites. Electron microscopy of CSL knock down parasites shows an altered cytostome morphology as compared to the control. Knock down parasites also exhibit less dense food vacuoles. If CSL plays a similar role to that of CIT1 in T. thermophila, this study could shed light on essential cytostome formation in malaria parasites.

296E A Secretory Phospholipid Binding GM2 Activator Protein is Essential for Cuticular Integrity in Caenorhabditis elegans NILUME FERDINAND NGALE1,2,3,5, STEPHEN GHOGOMU3, PHILIPPE POOLEVOORDE2, SIMON PICKARD4, NADIA MESSADDE4, LUC NEGRONI4, ARNAUD POTERSZMAN4, JACOB SOLOPOGU1Z, PATRICK LAUERT5, LUC VANHAMME4 1) University of California Merced; 2) Department of Molecular Biology, Institute of Biology and Molecular Medicine, IBMM, Université Libre de Bruxelles; 3) Molecular and Cell Biology Laboratory, Biotechnology Unit, University of Buea, Cameroon; 4) Department of Integrated Structural Biology, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France; 5) Laboratory of Neurophysiology, Institute of Biology and Molecular Medicine, IBMM, Université Libre de Bruxelles.

Nematodes are amongst one of the most successful phyla in the animal kingdom with adaptability in all known biological niches including ice poles, hot springs as well as up to 3.6 km beneath the earth’s surface. They can exist as free-living organisms but can also parasitize humans, livestock and plants where they cause an inordinate global disease burden. The cuticle, a tough and impermeable extracellular matrix mostly supports the nematode in its adaptability across different niches. The biogenesis and composition of the nematode cuticle is yet to be fully understood. In this study, using a combination of microscopy, biochemical, functional, structural and behavioral analysis, we report that the nematode orthologue of the GM2 activator protein (GM2AP) is an essential component for the integrity of the Caenorhabditis elegans cuticle. A transcriptional GFP reporter localized the expression of the GM2AP orthologue (cpp-1) predominantly in the hypodermal cells as well as the intestines. A GFP fused translational reporter identified cuticular expression of Cpp-1. Mutant C. elegans strains (OQ192) lacking the orthologue of the GM2AP (cpp-1) presented with increased cuticular permeability to Hoechst dye and could not survive in a hypotonic environment compared to wild type worms. The dauer stages of OQ192 were also found to be sensitive to SDS. Electron Microscopy analysis revealed a reduced cuticle in OQ192 strains as well as abnormal alae. The cuticle stages of OQ192 strains could not retain the Dil lipophilic dye as opposed to wild type (N2) strains. The Onchocerca volvulus orthologous protein, OvGm2AP expressed in Sf21 cells binds to lipids differentially on lipid overlay assay compared to a recombinant CPP-1 and the OvGm2AP CDNAs fully failed to rescue the permeability phenotype of OQ192 strains. Oxygen behavioral analysis assay revealed a close mid-body speed in OQ192 strains compared to N2 strains but much improved speed compared to tm3232 acs-20 mutant strains. Our results strongly suggest that the permeability phenotype observed in OQ192 strains arise from the lack of a lipid transport mechanism responsible for cuticular delivery of lipids. We conclude that CPP-1 is a hydromerally expressed phospholipid binding protein essential for cuticular integrity in C. elegans. We further suggest an evolution of the parasitic orthologues of CPP-1 in roles that support a parasitic lifestyle

297E When is a DNA Polymerase Not a Polymerase? Stephanie B. Delzell1, Scott Nelson2, Michele M. Klingbeil1 1) University of Massachusetts Amherst; 2) Iowa State University.

Mitochondrial DNA structure and replication in trypanosomes are essential for parasite survival and highly divergent from those in other eukaryotes. While most eukaryotes have one essential mitochondrial DNA polymerase, Trypanosoma brucei has three Pol I-like paralogues (POLIB, POLIC, POLID) that are independently essential to maintain their catenated kinetoplast DNA (kDNA) network. Preservation of these paralogues across kinetoplastids and diplonemids, and their non-redundant roles in T. brucei kDNA maintenance suggest these proteins have evolved specialized functions. Like many proofreading replicative DNA polymerases, POLIB and POLID contain polymerase and exonuclease domains. RNAi has established roles for POLIB and POLID in minicircle replication, but neither protein was enzymatically characterized to evaluate a high fidelity or supporting role in replication. Predictive modeling of POLIB revealed the thumb domain (369 amino acids) with homology to E. coli RNase T. To understand how this unique structure contributes to minicircle replication, we are characterizing POLIB enzymatic properties using recombinant His-tagged POLIB variants purified to near homogeneity. To simultaneously monitor polymerase and exonuclease activities, we used a fluorescently labeled DNA primer on DNA template. Wild type POLIB demonstrated robust 3′-5′ exonuclease activity that apparently outcompeted nucleotidyl incorporation. Exonuclease activity was detected on dsDNA overhang, blunt dsDNA and ssDNA templates, and these activities were ablated in the Exo- POLIB variant. Endonuclease activity was not detected. Gel shift assays using the POLIB variants revealed that the exonuclease domain is primarily responsible for DNA binding. Surprisingly, nucleotidyl incorporation was not detected in the Exo- POLIB variant. To further corroborate this finding, immunoprecipitation of epitope tagged Pol I-like proteins from T. brucei indicated that only POLID and POLIC exhibited the expected nucleotidyl incorporation while POLIB displayed only robust exonuclease activity. In the absence of detectable nucleotidyl incorporation, it is likely that the POLIB exonuclease activity is critical for minicircle replication and RNAi complementation studies are currently being used to further assess the role of the exonuclease domain.
Trypanosoma brucei gambiense, an extracellular protozoan parasite, is the primary causative agent of human African Trypanosomiasis. T. b. gambiense is endemic to West and Central Africa where it is transmitted to humans and animals by the bite of infected tsetse flies. In the bloodstream of an infected host, the parasite evades antibody recognition by altering a single Variant Surface Glycoprotein (VSG) that forms a dense coat on its cell surface. Each VSG has a variable N-terminal domain that is exposed to the host and a less variable C-terminal domain that is at least partially hidden from host antibodies. Our lab developed VSG-seq, a de novo VSG assembly method, to study VSG expression during mouse infections. This work revealed marked diversity in VSG expression within parasite populations in the animal model, but this finding has not yet been validated in a natural human infection. Here, we used VSG-seq to analyze VSGs expressed in the blood of twelve patients with a confirmed T. b. gambiense infection. The number of VSGs identified per patient ranged from one to twelve and, notably, two VSGs were shared among two or more patients. The N-terminal domain types (A and B) of the patient VSGs were determined using established predictive sequence models; B was the most predominant type among the forty-three VSGs in the dataset (79%). While the observations presented are limited in scope, they are the first indication of the number and diversity of VSGs in humans during infection and hint at a potential N-terminal domain type preference.

A forward genetic screen identifies genes essential for gametocyte development and transmission of the malaria parasite Plasmodium falciparum

Malaria transmission of the deadly parasite Plasmodium falciparum is mediated by mature sexual forms called gametocytes that represent an infection bottleneck. With restricted use of drugs targeting this stage, gametocytes propagate through the population unchecked, making it a prime target to block human to vector transmission. With recent progress in the identification of genetic factors linked to gametocyte commitment, genes essential for complete development and transmission remain largely unknown. Previous work in our laboratory achieved saturation-level mutagenesis of P. falciparum and obtained single disruption mutants (>38,000) with each gene being assigned a dispensability score for asexual blood-stage growth under ideal in vitro conditions. In this study, we evaluated scalable forward genetic screens to assess the likely importance of asexual dispensable genes in sexual-stage development. We performed our first high-throughput gametocyte screen using a pilot library of 128 unique piggyBac mutant clones and identified two distinct phenotypes that are ‘Advantageous’ or ‘Deleterious’ to gametocyte development. These genes are characterized further for their expression patterns, GO pathways, conservation with other Plasmodium spp. and other identifiable features. Our goal is to scale up to a 2000 mutant library and delineate the pathways and processes critical for the development and maturation of infectious male and female gametocytes. Through this study, we anticipate closing an important gap in the P. falciparum life cycle and lay the foundation for new antimalarial transmission-blocking intervention strategies.
There are 2 species in the genus: M. simplex (Kloss, 1966) and M. corvitellinum (Lacerda, Takemoto and Pavanelli, 2009). However, the morphological descriptions of the species many times are incomplete or unclear, and their genetic characterization has just recently started with a few sequences published belonging to M. simplex from Mexico. Adult and larval stages of specimens of this genus were found in different locations and hosts from Salta in northern Argentina. With the aim to determine whether they belong to one or multiple species of Magnivilletum, ITS1, 28S and COI DNA sequences of these samples are being analyzed in parallel to morphological and ecological studies. Using primers published for Macroderoididae (Al Khandari et al., 2011), a 300 bp fragment was obtained for the ITS1 region from cercariae DNA, while the 1200 bp expected sequence was obtained for the 28S gene from adults and cercariae using primers published for M. simplex from Mexico (Hernandez Menas et al., 2016). No specific sequences were obtained using different sets of primers for COI. Maximum likelihood trees with 1000 bootstraps (MEGA X) of the 28S sequence showed that the specimens found in Salta grouped close to M. simplex from Mexico, but the 1.45% difference in their sequence identity suggests that they belong to a different species. Similar results were obtained with the ITS1 fragment, with the individuals from Salta grouping alone in a branch between Alloglossiadii sp. and Pilagorias sp. The lack of results for the COI gene highlights the absence of nucleotide sequences in GenBank for close relatives of Magnivilletum, and in general, the need of more studies on species from South America to widen the range of genetic sequences available for comparisons. Together with ecological and morphological data, these results suggest that the specimens of Magnivilletum found in Salta, Argentina belong to a new species.


304E In silico design of Eimeria tenella vaccine candidates. Tean Zaheer1, Raozid Abbas1, Muhammad Imran1, Sajjad ur Rahman2, Iqra Zaheer3, Tariq Javid4 1) University of Agriculture, Faisalabad; 2) Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan; 3) Department of Pathology, University of Agriculture, Faisalabad, Pakistan; 4) Medi-Excel Pharmaceuticals, Islamabad, Pakistan.

Coccidiosis is one of the most important parasitic diseases of poultry worldwide. It accounts for more than 3 billion dollars spent on prevention, treatment and control of Eimeria. Various prevention and control methods have been adopted as anti-coccidial, but the parasite is still a menace to the conventional and semi-conventionally built poultry houses. Eimeria (E.) tenella is one of the most significant species, leading to ‘bloody coccidiosis’, making it an important target for vaccine development. The genetically diverse nature of the parasite makes it difficult to test and control at farm levels. Moreover, many vaccine candidates have been tested for their efficacy and many have been commercialized as cocktail vaccines. This study employs the use of computer-assisted determination of vaccine candidates for E. tenella, that had not been experimented before. The B and T-cell multi-epitope vaccine candidates proposed in this study were predicted on the basis of high degree of conservation, localization analyses and local isolate sequences from the Asia region. Based on available databases, the vaccine candidates were refined. The physicochemical properties, molecular structures and antigenic potential of candidates were predicted with and without the addition of adjuvants. The epitopes have been shown to possess <65% identity to available chicken or its microbeirome sequences. The proposed vaccine candidates could be further trialed within in vitro and in vivo studies for the cross-protective efficacy and potential vaccine candidate.

305E Decoding combinatorial patterns of histone posttranslational modifications of Plasmodium falciparum. Hilde von Gruning1, Mariel Coradin2, Mariel G. Mendoza2, Benjamin A. Garica2, Lyn-Marie Birkholtz1 1) University of Pretoria, Pretoria, South Africa; 2) University of Pennsylvania, Philadelphia, PA, USA.

Histone post-translational modifications (PTMs) dynamically regulate chromatin structure which accompanies specified biological outcomes for eukaryotic cells. There is increasing evidence of histone PTM crosstalk, which is a phenomenon that in combination can act synergistically as a mechanism of gene regulation. Here, we investigate the combinatorial histone code of in both the transmissible sexual stages as well as the proliferative, clinically relevant asexual stages of the P. falciparum parasite and aim to provide evidence that these modifications of histone PTMs result in functionally distinct outcomes. We quantify and map combinatorial histone PTMs on histone H3 for P. falciparum parasites for three asexual and gametocyte developmental stages. Using advanced quantitative middle-down mass spectrometry (MS), the dynamic and complex patterns of histone PTM combinations occurring in three developmental stages was revealed, which suggests an alternative higher order mode of gene regulation in the parasite. Using chromatin immunoprecipitation (ChIP) coupled with high-resolution MS, we identify the proteins associated with known and novel histone PTMs in the asexual trophozoite, immature and transmissible mature gametocyte stages. This revealed previously associated proteins in addition to a unique subset of proteins enriched for metabolic enzymes. We are in the process of using chemoproteomic (click chemistry/pulldown) approaches to identify protein target(s) of these compounds. We then hope to confirm binding and further characterize the target(s).

305E Deciphering Magnivilletum sp. (Digenea: Alloglossiidae) from Salta, Argentina, with genetic markers. Carolina Davies1, 2, Juan José Lauthier3, José Saravia4, Florencia Liquin1, 5, Héctor Cristobal1, 4, Dora Ana Davies5 1) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; 2) Instituto de Patología Experimental-Universidad Nacional de Salta (UNSA), Argentina; 3) Parasitology Department, Kochi Medical School, Kochi University, Japan; 4) Facultad de Ciencias Naturales (FCN)-UNSA, Argentina; 5) Instituto de Investigaciones para la Industria Química (INIQIU)-CONICET-UNSa, Argentina.

The genus Magnivilletum has been reported in the Neotropical region parasitizing characiform, siluriform and cyprinodontiform fish. To date, traditional ecological and morphological studies showed that there are 2 species in the genus: M. simplex (Kloss, 1966) and M. corvitellinum (Lacerda, Takemoto and Pavanelli, 2009). However, the morphological descriptions of the species many times are incomplete or unclear, and their genetic characterization has just recently started with a few sequences published belonging to M. simplex from Mexico. Adult and larval stages of specimens of this genus were found in different locations and hosts from Salta in northern Argentina. With the aim to determine whether they belong to one or multiple species of Magnivilletum, ITS1, 28S and COI DNA sequences of these samples are being analyzed in parallel to morphological and ecological studies. Using primers published for Macroderoididae (Al Khandari et al., 2011), a 300 bp fragment was obtained for the ITS1 region from cercariae DNA, while the 1200 bp expected sequence was obtained for the 28S gene from adults and cercariae using primers published for M. simplex from Mexico (Hernandez Menas et al., 2016). No specific sequences were obtained using different sets of primers for COI. Maximum likelihood trees with 1000 bootstraps (MEGA X) of the 28S sequence showed that the specimens found in Salta grouped close to M. simplex from Mexico, but the 1.45% difference in their sequence identity suggests that they belong to a different species. Similar results were obtained with the ITS1 fragment, with the individuals from Salta grouping alone in a branch between Alloglossiadii sp. and Pilagorias sp. The lack of results for the COI gene highlights the absence of nucleotide sequences in GenBank for close relatives of Magnivilletum, and in general, the need of more studies on species from South America to widen the range of genetic sequences available for comparisons. Together with ecological and morphological data, these results suggest that the specimens of Magnivilletum found in Salta, Argentina belong to a new species.


304E Deciphering Magnivilletum sp. (Digenea: Alloglossiidae) from Salta, Argentina, with genetic markers. Carolina Davies1, 2, Juan José Lauthier3, José Saravia4, Florencia Liquin1, 5, Héctor Cristobal1, 4, Dora Ana Davies5 1) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; 2) Instituto de Patología Experimental-Universidad Nacional de Salta (UNSA), Argentina; 3) Parasitology Department, Kochi Medical School, Kochi University, Japan; 4) Facultad de Ciencias Naturales (FCN)-UNSA, Argentina; 5) Instituto de Investigaciones para la Industria Química (INIQIU)-CONICET-UNSa, Argentina.

The genus Magnivilletum has been reported in the Neotropical region parasitizing characiform, siluriform and cyprinodontiform fish. To date, traditional ecological and morphological studies showed that there are 2 species in the genus: M. simplex (Kloss, 1966) and M. corvitellinum (Lacerda, Takemoto and Pavanelli, 2009). However, the morphological descriptions of the species many times are incomplete or unclear, and their genetic characterization has just recently started with a few sequences published belonging to M. simplex from Mexico. Adult and larval stages of specimens of this genus were found in different locations and hosts from Salta in northern Argentina. With the aim to determine whether they belong to one or multiple species of Magnivilletum, ITS1, 28S and COI DNA sequences of these samples are being analyzed in parallel to morphological and ecological studies. Using primers published for Macroderoididae (Al Khandari et al., 2011), a 300 bp fragment was obtained for the ITS1 region from cercariae DNA, while the 1200 bp expected sequence was obtained for the 28S gene from adults and cercariae using primers published for M. simplex from Mexico (Hernandez Menas et al., 2016). No specific sequences were obtained using different sets of primers for COI. Maximum likelihood trees with 1000 bootstraps (MEGA X) of the 28S sequence showed that the specimens found in Salta grouped close to M. simplex from Mexico, but the 1.45% difference in their sequence identity suggests that they belong to a different species. Similar results were obtained with the ITS1 fragment, with the individuals from Salta grouping alone in a branch between Alloglossiadii sp. and Pilagorias sp. The lack of results for the COI gene highlights the absence of nucleotide sequences in GenBank for close relatives of Magnivilletum, and in general, the need of more studies on species from South America to widen the range of genetic sequences available for comparisons. Together with ecological and morphological data, these results suggest that the specimens of Magnivilletum found in Salta, Argentina belong to a new species.


Global malaria control and elimination efforts rely on new generations of compounds with novel modes of action against the malaria parasite *Plasmodium falciparum*. Recently, in vitro evolution experiments have identified mutations in the parasite’s Acetyl-CoA Synthetase (PF3D7_0627800; PfAcAS) which confer resistance to structurally distinct compounds, including MMV084978 and MMV019721. Allelic exchange using the CRISPR/Cas9 system confirmed that the A597V or T648M mutations in PfAcAS phenocopied the resistance phenotype. Conditional knockdown using the Tet-D0ZI aptamer system demonstrated that PfAcAS is essential for parasite growth, and partial knockdown sensitized parasites to both compounds. MMV019721 and MMV084978 directly inhibited recombinant PfAcAS activity in a substrate-competitive manner, with Ki values of 73 and 369 nM respectively, and the A597V mutation reduced inhibitor affinities by more than 90-fold. Orthologues of PfAcAS in eukaryotes catalyze the formation of the central metabolite acetyl-CoA from acetate, coenzyme A and ATP, and participate in a range of essential processes including epigenetic regulation. Metabolomic analyses revealed that exposure of trophozoite parasites to PfAcAS inhibitors reduced cellular acetyl-CoA levels by ~4-fold. To further investigate the biological implications of PfAcAS inhibition, western blot analyses were conducted to examine histone acetylation changes following brief exposures to MMV019721, MMV084978 and other PfAcAS inhibitors. Significant reductions in the acetylation of H3 and H4 histones at the H3K9, H4K8 and H4ac4 sites were observed for all inhibitors in wildtype but not in resistant parasites (ANOVA, p < 0.05). Knockdown of PfAcAS expression by 70% also reduced acetylation of H4K8 and H4ac4 markers by 92% and 40% respectively (T-test p < 0.05). Together these findings suggest that PfAcAS may play a role in maintaining nucleo-cytoplasmic acetyl-CoA pools that are necessary for the epigenetic regulation of parasite gene expression. These findings highlight inhibitors of PfAcAS as promising drug targets with inhibitors which exert their activity against the malaria parasite by a unique mode of action.

### 307E Study of gibbon APOL1-like apolipoprotein lytic activity in *Trypanosoma brucei* and mammalian cells

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Trypanosoma brucei subspecies and primates have undergone a co-evolutionary arms race that has resulted in the emergence of host defense mechanisms against the parasites. Serum of humans and some old-world primates is lytic to most species of animal-infective trypanosomes. This property is attributed to a subset of high-density lipoproteins (HDL) known as trypanosome lytic factors (TLF). The trypanolytic component of TLF complexes is Apolipoprotein L1 (APOL1). APOL1 is a cation channel-forming protein that allows movement of cations through membranes. APOL1 is cytolytic when overexpressed in mammalian cells, with the exception of hepatocytes, which secrete it onto HDL complexes. Secreted HDL-bound APOL1 has been identified in humans and other primates, such as gorillas, baboons, sooty mangabeys, and mandrills. Chimpanzees and bonobos have deleted the orthologous APOL1 gene. Here, we studied an APOL1-like gene in gibbons, another primate from the Hominioidea clade. Gibbons are distributed throughout Asia and despite their arboreal habits they could be under selective pressure by African trypanosomes such as T. evansi. Gibbons have a full-length gene named APOL2.1 (gbAPOL2.1) containing a signal peptide, which may facilitate secretion onto HDL complexes. gbAPOL2.1 has 68% amino acid homology to human APOL1 (hAPOL1) and 67% to gorilla APOL1.

Using mice transiently transfected with plasmid DNA encoding for either gbAPOL2.1, gAPOL1 or hAPOL1, we compared their trypanolytic activity. Sera were isolated and the HDL fraction was purified. Immunoblotting assays results revealed that gAPOL1, hAPOL1, and gbAPOL2.1 proteins were produced. Transfected mice were challenged with T. brucei to test the trypanolytic activity of gbAPOL2.1, gAPOL1, and hAPOL1 in vivo. Mice producing hAPOL1 and gbAPOL1, but not gbAPOL2.1, were protected from *T. brucei* infection, suggesting that gbAPOL1 is not trypanolytic in vivo. Next, we tested the cytotoxicity of these APOL proteins when expressed in immortalized human embryonic kidney cells (HEK293). Similar to the trypanolytic assay, HEK293 cells producing hAPOL1 and gAPOL1, but not gbAPOL2.1, were lysed. These results suggest that despite amino acid sequence homology to hAPOL1, gbAPOL2.1 is not lytic to *T. b. brucei* and mammalian cells, suggesting that specific amino acid residue differences results changes in the secondary structure of gbAPOL2.1, which could explain the absence of lytic activity.

### 308E Optimisation of a Loop-Mediated Isothermal Amplification (LAMP) assay: improving the performance of the reaction in diagnostics of *Schistosoma mansoni* in human and snail samples

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Schistosomiasis mansoni is a snail borne parasitic disease associated to poverty and low sanitation condition. It is estimated that 7.1 million people are infected in Latin America and 95% of them live in Brazil. The cost of the disease per year in Brazil is estimated in US$ 41.7 million, highlighting its relevance in public health and economics. Therefore, better strategies to control and eliminate schistosomiasis in Brazil are strongly needed. The association between proper diagnosis and early treatment is an important measure to control disease, but diagnostics improvements are necessary in order to detect the infection even in low burden conditions. Besides that, the World Health Organisation also recommends mapping and monitoring snails breeding sites in endemic areas to detect active transmission spots. Isothermal amplification methods, such as the loop-mediated isothermal amplification (LAMP), seem to be a good alternative for a more sensitive, rapid, and cost-effective diagnostic. However, to standardize this assay with a high specificity and sensitivity is a challenge in regard of the variety of parasites that are sympatric with *Schistosoma mansoni* and the occurrence of different intensity and endemicity of the disease in distinct areas. This work aimed to optimize a LAMP assay for *S. mansoni* diagnostics recently published using different types of hosts’ samples (*Biomphalaria* snails and human’s urine and faeces) to obtain higher specificity considering the incidence of other common parasites. We found that with minor changes in the protocol, the LAMP assay was efficient in detecting *S. mansoni* infection in urine and stool samples as well as in snails with no cross-reactivity with other helminths (e.g. *Trichuris trichiura*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Ancylostomids* and different trematodes that also parasites *Biomphalaria* snails). The sensitivity was accessed by the limit of detection of the reaction and the assay was able to detect less than the equivalent to two parasites’ cells, considering that the whole *S. mansoni* genome has around 580fg of DNA. Therefore, we state that LAMP is a good alternative for schistosomiasis mansoni diagnostic in both humans’ urine and faeces, and also to monitor potential and active infection spots by the intermediate host exam. Our findings will be further validated using field samples.

### 309E Multiplex species-specific isothermal DNA amplification for rapid molecular detection of viscerotropic and dermotropic *Leishmania* species in Brazil

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Recent studies revealed that dermotropic *Leishmania* species can be found causing visceral leishmaniasis and the opposite is also true, when classically viscerotropic species are found in cutaneous lesions. It represents a problem for leishmaniasis epidemiology and a challenge to better understand dynamics of leishmaniasis physiopathology. Loop-mediated isothermal amplification (LAMP) is an useful alternative molecular diagnosis method to detect *Leishmania* DNA, however, the targets used for that are not specific and virtually can detect any species in *Leishmania* genus. Here we develop a LAMP-based diagnostic method to specifically identify *L. infantum*, *L. braziliensis* and *L. amazonensis*. Based on a pan-genome approach, we selected species-specific targets among 26 publicly available *Leishmania* genomes, including at least, 15 different species. The accessory genome was filtered to yield unique species-specific sequences. The selected targets codes for unique hypothetical proteins. LAMP primers were designed using the software primer explorer
Polarization. We will be utilizing a DNA binding domain from the Apicomplexan Apetala 2 (ApiAP2) proteins and a fluorescently labeled DNA probe. If the labeled accomplish this, we envision three potential transcription inhibition screens. The first assay measures inhibition of protein-DNA interaction previously engineered *P. falciparum* uracil phosphoribosyltransferase (FCU). FCU-expressing parasites can salvage synthetically labeled uracil for incorporation into nascent mRNA. We will detect transcription inhibition by measuring the incorporation of Ethynyl-uracil (5-EU) labeled post-salvage via Click chemistry with fluorescent azide. To verify 5-EU can the polymorphic region TNFα polymorphisms and GRA6 serotypes correlated with specific clinical manifestations and immune response in ocular toxoplasmosis.

12), IL-17R, TLR-

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Our analysis of the genomes of different kinetoplastid species revealed the presence of additional PGK isoenzymes. Some open reading frames that such as *T. brucei* replication. PGK has been studied in a wide variety of organisms, including protists. In kinetoplastids, groups of protists that include parasites of clinical interest, gluconegogenes and centrosome duplication. The third kinase, MAPK2, is specific to and conserved throughout Alveolata, though its function is unknown. We used the auxin-inducible degron system to determine phenotypes associated with MAPK2 loss-of-function in Toxoplasma. We found that parasites lacking MAPK2 were unable to complete DNA replication, and arrested before mitosis starts. While the parasites continued to replicate their mitochondria and apicoplasts, and to elongate and split the Golgi apparatus, they failed to duplicate both centromosomal cores at the basal end of the nucleus and initiate daughter-cell budding, which ultimately led to parasite death.

The failure in centromosal cores duplication is distinct from the MAPK1 phenotype. As we did not observe MAPK2 localization at the centrosome at any point in the cell cycle, our data suggest MAPK2 regulates a process at a distal site that is required for completion of centrosome duplication.

Phosphoglycerate kinase (PGK) is an enzyme conserved in all three domains of life. In glycolysis, this enzyme catalyzes one of the two ATP-producing reactions through the reversible conversion of 1,3-bisphosphoglycerate (1,3BPG) to 3-phosphoglycerate (3PGA). Furthermore, it also participates in gluconeogenesis in the opposite reaction to produce 3PGA and ADP. Among the glycolytic enzymes, PGK is one of the glycolytic enzymes, which has been identified as having also moonlighting functions because of its participation in functions not associated with energy metabolism. Moonlighting glycolytic enzymes have been found to be involved in processes such as cell invasion, DNA replication and repair in the nucleus, induction of tumorigenesis, autophagy, and viral replication. PGK has been studied in a wide variety of organisms, including protists. In kinetoplastids, groups of protists that include parasites of clinical interest, such as *T. brucei, T. cruzi* and *L. major*, various isoenzymes have previously been identified to which functions mainly related to carbohydrate metabolism are attributed. Our analysis of the genomes of different kinetoplastid species revealed the presence of additional PGK isoenzymes. Some open reading frames that encode these PGKs are unusually large. Products of some of these genes appear to contain regulatory module domains in their structure, a characteristic that can confer functional diversity to the PGK isoenzymes in these kinetoplastids. Additionally, some products of candidate PGK genes are catalytically inactive, they are "dead" enzyme genes since they lack essential residues to carry out their catalytic reaction. Understanding the functional diversity found in kinetoplastid PGK isoenzymes will contribute to understanding new roles in the physiological context of these parasites.

In human ocular toxoplasmosis, serotype is related with greater severity. We analyzed Toxoplasma GRA6 serotype in 23 patients with ocular toxoplasmosis (13 confirmed, two co-infections- and eight unconfirmed cases) and 20 individuals chronically infected with *Toxoplasma* but without ocular involvement. In patients with ocular toxoplasmosis, we also studied host gene polymorphisms related to immune response (IL-1β; IL-1α; IL-10; IFN-γ, TNF-α, IL-12), IL-17R, TLR-9, and P2RX7. Additionally, eight patients were studied for the production of TNFa, IL-1β, IFN-γ and IL-10 by their peripheral leukocytes after ex vivo stimulation with soluble *Toxoplasma* antigens. There were no differences in the distribution of serotypes (GRA6-I versus GRA6 non-I) between infected individuals with- or without ocular involvement. Seropositivity for GRA6-I was associated with higher number of retinal lesions and higher levels of IL-1β. Two polymorphisms were associated with specific clinical manifestations of OT. IL-10 -819 C/T with bilateral lesions and IL-12 +169774 A/C with synchiae. Higher levels of IL-10 were found in patients with the allele G/G at the polymorphic region IL-10 -1082. People with a GRA6 I serotype and possessing the allele G/G at the polymorphic region TNFa-857 suffered from an increased number of retinal lesions. We found a positive association between host cytokine genes polymorphisms and GRA6 serotypes correlated with specific clinical manifestations and immune response in ocular toxoplasmosis.

Novel drug targets are crucial to combat drug-resistant malaria parasites as they arise in the field. High-throughput screens of small molecule compound libraries have been used successfully for identifying new candidate antimalarials. However, these screens do not allow for the identification of the compound’s mode of action. Additional screens using biological-process-specific assays are therefore valuable to accelerate hit-to-lead optimization. We aim to explore and optimize three potential assays for the characterization of compounds that specifically perturb transcription in *Plasmodium falciparum* parasites.

To accomplish this, we envision three potential transcription inhibition screens. The first assay measures inhibition of protein-DNA interaction in vitro via fluorescent polarization. We will be utilizing a DNA binding domain from the Apicomplexan Apetala 2 (ApiAP2) proteins and a fluorescently labeled DNA probe. If the labeled probe is competed away by inhibiting compounds, dissociation will be detected by the emission of depolarized light. Our second approach is a stage-specific in vivo screen. Here, green fluorescent protein (GFP), under control of an ApiAP2 regulated stage-specific promoter, will act as a reporter for transcriptional activation. The third approach is an in vivo assay for measuring nascent RNA transcription via the incorporation of a synthetically labeled uracil. Our lab has previously engineered *P. falciparum* parasites capable of pyrimidine salvage by the expression of a bifunctional yeast fusion gene of cytosine deaminase, and uracil phosphoribosyltransferase (FCU). FCU-expressing parasites can salvage synthetically labeled uracil for incorporation into nascent mRNA. We will detect transcription inhibition by measuring the incorporation of Ethynyl-uracil (5-EU) labeled post-salvage via Click chemistry with fluorescent azide. To verify 5-EU can be salvaged by the parasite, we treated FCU parasites with atovaquone, which inhibits de novo RNA synthesis, leading to death, but would be rescued upon the
salvage of environmental S-EU. Our preliminary results indicate that S-EU is readily salvaged by the parasite and is incorporated into the RNA biomass. All three proposed assays are compatible with a plate-well format for high throughput screening for inhibitors of transcription.

314E Long-term live-microscopy of Cryptosporidium reveals direct development of sexual stages Elizabeth English1, Jayesh Tandel1, Boris Striepen1 1) University of Pennsylvania.

Cryptosporidium is the second leading cause of pathogen-associated diarrhea in children under the age of two. There is no vaccine against Cryptosporidium infection, and the only approved drug (nitazoxanide) is not effective in immunocompromised or malnourished patients. The Cryptosporidium life cycle appears relatively simple compared to other members of the Apicomplexan phylum, as both asexual and sexual reproduction occurs within a single mammalian host. Infection is established following ingestion of oocysts, which contains four sporozoites that invade epithelial cells of the small intestine. Parasites replicate asexually for several generations before differentiating into sexual stages. Asexual parasites undergo three rounds of nuclear division, followed by cytokinesis to generate eight merozoites. A second asexual stage called a type II meront, which produces only four merozoites, is believed to precede sexual differentiation occurring in male and female micro- and macrogametes. We established conditions for live microscopy that allow imaging over multiple days. Using transgenic parasites with a fluorescently tagged histone, we observe, track, and compare the nuclear division of asexual and sexual parasite stages. We establish the length and sequence of the cell cycle of asexual and male parasites and we map the developmental fate of parasites across multiple rounds of invasion and egress. We make three important and surprising observations. 1. Development to gametes is direct from type I meronts and we find no evidence for the existence of a type II meront. 2. The progeny of type I meronts appears collectively committed to a sexual or asexual fate. 3. Meronts committed to a sexual fate give rise to both sexes.

315E Dissecting the Unique Invasion Machinery of Apicomplexan Parasites Li-av Segev Zarko1, Peter Dahlberg1, Daniel Peltz2, Stella Sun1, Wah Chiu1, James Sethian2, John Boothroyd1 1) Stanford University, Stanford, CA; 2) University of California Berkeley, Berkeley, CA.

The phylum Apicomplexa includes several of the most prevalent and important human eukaryotic pathogens, such as the malaria-causing Plasmodium and Toxoplasma gondii that can cause severe neurological disease in the developing fetus and those who are immunocompromised. These intracellular parasites enter a host cell by deploying a remarkable machine at their anterior end known as the apical complex (AC), for which the phylum is named. At the start of the invasion, the AC, including a unique spiral of tubulin-based fibrils called the conoid, is protracted and secretion events occur from two types of distinct secretory organelles, capsule-shaped micronemes and club shaped rhoptries. The exact means by which these various components of the AC coordinate invasion has been a largely mysterious, in part, to a lack of tools capable of resolving the structure of this extraordinary apparatus in its natural context. We are developing a pipeline to study the complex and dynamic structure of the AC in parasites devoid of any chemical fixation using cryo-electron tomography. Tomography reconstruction displays the 3D organization of subcellular organelles while computational analysis of the data provides information on the biomolecular composition at near atomic resolution. Our images of extracellular Toxoplasma tachyzoites reveal a possible interaction between the enigmatic apical vesicles that are aligned along the intra-conoid microtubules with a tip of a rhoptry only under calcium flux, a stimulus for activation of the invasion machinery in this phylum of parasites. Annotation of the reconstructed tomograms using mixed scale convolutional neural network is used to identify and extract the volumes of the subcellular organelles and study their characteristics and function during invasion.

316E mRNA localization: a possible role in the regulation of stage-specific gene expression during Trypanosoma cruzi development Karina Sabalette1, Vanina Campol1, Javier De Gaudenzi1 1) IIBIO-UNSAM.

Trypanosoma cruzi, the causative agent of Chagas disease, is characterized by regulating its gene expression mainly at post-transcriptional level. RNA regulators consist of riboncuble-binding complexes having mRNAs whose protein products act cooperatively in a particular biological pathway. These clusters are regulated by one or more RNA-binding proteins (RBPs), thereby enabling quick changes of the protein cellular profile in response to internal or external stimuli. Previous results demonstrated that the small trypanosome-exclusive protein U-rich RBP 1 (TcUBP1) is involved in metabologeny and exerts its function through the interaction with numerous mRNAs encoding cell-surface glycoproteins preferentially expressed in the tripeomastigote infective stage, including members of the transialidase and trans-sialidase-like (TcS) multigenic family. The aim of this study was to evaluate if mRNA localization is a mechanism for stage-specific gene regulation. Using RNA FISH with a specific Cy3-oligo probe for TcS transcripts, we observed that over-expression of TcUBP1-GFP in epimastigotes resulted in changes in the localization of these mRNAs from the posterior region to the peri-nuclear region of the cell, as is typically observed in tripeomastigotes. To get a deep insight into this result we used the wild-type T. cruzi CL-Brener strain and performed a trypeomastigote-to-epimastigote differentiation in vitro. In this case we observed the relocalization of the transcripts from peri-nuclear region to the posterior region, and the same change was observed for transcripts from another cell-surface protein family named TAVS, highly expressed in Tripeomastigotes. Indirect immunofluorescence labeling of epimastigote cells with an anti-TcRas antibody reveals an organelle absent in infective stages. The results obtained suggest that RNA mobilization appears to operate in the regulation of stage-specific expression, where the reservosome could play a role in storing and protecting the mRNAs during the epimastigote replicative stage. Finally, analyzing bioinformatic tools, we focused on putative cis-acting RNA regulatory elements located in both TcS and TAVS 3’UTRs and identified novel interacting candidates involved in this mRNA translation.

317E Probing the role of MFR4 in Halofuginone Resistance and the Adaptive Proline Response Akansha Pant1, Amy Deik2, Claudia Taccheri1, Lola Fabgami1,3, Mark Tye3,4, Selina Bopp1, Clary Clish2, Ralph Maitzschek1,2,3,4, Amanda Lukens1,2, Dyann Wirth1,2 1) Harvard TH Chan School of Public Health, Boston,MA,USA; 2) The Broad Institute, Cambridge, MA, USA; 3) Harvard University, Cambridge, MA, USA; 4) Massachusetts General Hospital, Boston, MA, USA.

Halofuginone (HFG) is a potent antimarial that targets the P. falciparum cytosplasmic prolyl tRNA synthetase (PfPRS). Previously, we showed that parasites selected for an extended time with increasing concentrations of HFG can develop high-level resistance conferred by mutations in the PfcPRS target. In contrast, short-term exposure with HFG yielded parasites with a moderate resistance profile yet to mutation in the pfprs locus. The exposure to HFG triggers a novel mechanism of drug tolerance, termed the Adaptive Proline Response (APR), wherein intracellular proline levels increase twenty-fold. This metabolic adaptation persists after drug withdrawal and renders parasites resistant to HFG. When we investigated the metabolic source of the elevated proline, we found that proline derived from arginine was a major contributor to the APR. We then assessed the requirement of the arginine to proline pathway for the APR by generating ornithine δ-aminitransferase knockout (Dd2-DAT) parasites. These parasites were unable to generate proline from arginine nor activate the APR. Despite this, a clone was isolated that tolerated HFG. Whole genome sequencing of these parasites identified mutations in the mfr4 gene (PF3D7_0914700) likely resulting in gene function of loss. MFR4 belongs to the major facilitator superfamily of transporters which remain largely uncharacterized in P. falciparum. To understand the contribution of MFR4 to HFG-resistance, we used CRISPR/Cas9 to disrupt the locus and generate MFR4 knockout parasites (Dd2-ΔMFR4). Interestingly, Dd2-ΔMFR4 parasites showed a twenty-fold increase in HFG dose-response relative to wild-type (Dd2 EC50 = 0.7nM, Dd2-ΔMFR4 EC50 = 14nM). However, these parasites were never exposed to the HFG before. To further understand the role of MFR4 on intracellular proline levels, we are conducting metabolomics studies in the Dd2-ΔMFR4 knockout parasites. Future efforts aim to unravel the underlying mechanisms involving putative transporter proteins and exploit them for drug discovery.

318E Robotic Plasmodium sporozoite extraction from Anopheles mosquito salivary glands Tessa Seltzer1, Zephyr Pitré1, Christopher Sutanto1, Samantha Brown1, Thurston Herrick1, Kamalakannan Vijayan1, Elizabeth Glennon1, Natasha Bourgeois1,2, John Altschinson1,3, Alexis Kaushansky1,2,3,4,5 1) Center for
Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA; 2) Department of Global Health, University of Washington, Seattle, WA; 3) Department of Pediatrics, University of Washington, Seattle, WA; 4) Brotman Baty Institute, Seattle, WA; 5) Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA.

Malaria remains a substantial worldwide health problem. The *Plasmodium* parasite, the causative agent of malaria, develops into its human-infectious form in the salivary glands of female *Anopheles* mosquitoes. Hand dissections of these salivary glands are necessary to perform research on *Plasmodium* transmission and initial development within the liver of the human host and are also required for production of promising malaria vaccine candidates that rely on the generation of live parasites. The process of hand dissection is laborious for research and small to medium clinical trials, and it is virtually untenable to increase production to the scale needed for worldwide administration. Here, we describe the development of two manufactured robotic systems, which dramatically streamline the isolation of *Plasmodium* parasites from *Anopheles* salivary glands. The Squito Splitter, an automatic dissector, separates the mosquito head and thorax from its abdomen. The Squito Splitter, an automatic dissector, processes this material to isolate parasites. These devices can be manufactured using standard milling and lathe work, 3D printing, and basic circuits. Our prototype devices isolate parasites which have been shown to be infectious in vitro, and do not introduce additional bacterial contamination compared to hand-dissected mosquitoes. Together, our robotic systems have the potential to simplify the process of performing *Plasmodium* research for the scientific community and may streamline the process of generating malaria-protective vaccines.

319E Knockdown of EXP2 in the *Plasmodium* liver stage suggests major differences from blood stage function

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Malaria parasites develop within both hepatocytes and erythrocytes during vertebrate infection. To create a niche for survival in these remarkably diverse host cell types, the parasite exports a repertoire of effector proteins to remodel the host cell, a process that requires translocation across the parasitophorous vacuole (PV) in which the parasite dwells. This event has been extensively studied in the blood stage where it is facilitated by the *Plasmodium* Translocon of Exported proteins (PTEX). The core PTEx complex is composed of the membrane pore EXP2, the AAA+ chaperone unfoldase HSP101 and PTEX150, which forms a flange-like adaptor that docks HSP101 onto the pore. In addition to its role in protein export, EXP2 also functions in small molecule transport across the vacuole membrane. While not well characterized, protein export is also expected to occur in the liver stage to mediate hepatocyte subversion. EXP2 has been shown to be important for efficient liver stage development into the blood stage, however its function in the infected hepatocyte remains unclear. Curiously, while EXP2 and PTEX150 are both present at the liver stage PV, previous studies could not detect HSP101 by fluorescence microscopy. Using a proximity-labeling proteomic strategy, we were similarly unable to detect HSP101 in the liver stage. These observations indicate major differences in the cellular export machinery and the role of EXP2 and PTEX150 function during hepatocyte infection. Here, we employed the glucosamine-activated glmS ribozyme, a common approach for conditional gene knockdown in the *Plasmodium* falciparum blood stage, to study EXP2 function during *Plasmodium berghei* liver stage development. Insertion of the glmS sequence into the *exp2* 3'UTR enabled robust knockdown at concentrations of glucosamine found to be non-toxic to host hepatoma cells. Surprisingly, depletion of EXP2 did not apparently impact liver stage parasite development as assessed by parasite size and morphology, in striking contrast to the blood stage where loss of EXP2 results in catastrophic arrest and death early during intraerythrocytic development. Our findings suggest major differences in EXP2 function between blood and liver stages and extend the utility of the glmS ribozyme to study liver stage protein function.

320E Comparative Analysis of Features that Contribute to CNV Formation in Various *Plasmodium* Species


Genome amplifications, a type of DNA copy number variation (CNV), are a common method of adaptation of *Plasmodium falciparum* in response to drug treatment and other selective factors. Of the *Plasmodium* species, *P. falciparum* in particular is astoundingly A/T-rich and we predict that this unique genomic characteristic may serve an evolutionarily conserved and functional purpose. We previously detected long monomeric A/T tracks at the breakpoints of *P. falciparum* resistance-conferring CNVs. Additionally, we identified nearby, but distinct A/T-rich sequences that are predicted to form DNA hairpins an encourage DNA breaks. Based on these observations, we proposed a “trigger site” model in which long monomeric A/T tracks are utilized to repair DNA breaks by an error-prone, microhomology-mediated pathway that encourages the generation of novel CNVs. We applied this model and our analysis pipeline to other *Plasmodium* species and found that long monomeric A/T tracks were enriched within *Plasmodium* genomes regardless of their overall genome A/T content. Furthermore, we analyzed known CNVs in two other *Plasmodium* species, *P. vivax* and *P. knowlesi*, and found long monomeric A/T tracks at CNV breakpoints as well as nearby stable DNA hairpins. The evolutionary conservation of AT-richness and the overrepresentation of “trigger site” features at CNVs in different *Plasmodium* species reinforce our model and stress the need for further investigation of the molecular mechanisms of *Plasmodium* CNV creation.

321E : Molecular Characterization of *Toxoplasma gondii* in Pregnant Women from Mali and Nigeria

*Henrietta Awobode1, Mazo Koné1,2* 1) University of Ibadan, Nigeria; 2) Pa & Ka Medical laboratory, Bamako, Mali.

**Background:** *Toxoplasma gondii* has a clonal population structure and three clonal lineages are predominant, although many more strains have been reported. Genetic variation in this parasite can result in recombinants or atypical strains. This study aims to determine the strains of *Toxoplasma gondii* isolates indigenous to Mali and Nigeria and their genetic variation.

**Methods:** A cross-sectional study was conducted in Ibadan South East Local Government Area, Ibadan, Nigeria at five Primary Health Centres and also at the Papa and Kadiatou Medical Laboratory in Bamako, Mali between April 2017 and June 2018. Peripheral blood (130) collected from pregnant women during anti-natal visits was screened for IgG and IgM antibodies to *Toxoplasma gondii*. The parasite 529 bp repetitive element was amplified by PCR from DNA of 18 seropositive samples. Isolates were sequenced and analysed.

**Results:** Seropositivity to anti-*Toxoplasma gondii* IgM was 12.3% (16/130), and 90% (117/130) to IgG. Analysis of the Nigerian (KTGS) and Malian (KTG6, KTG7 and KTG8) parasite isolates showed they were recombinants of the RH reference strain. Sequence analysis showed that the KTG isolate belonged to both the KTG7(Mali) and AF146527(RH) which were the major and minor parents respectively. The KTG6 sequence showed a 98% similarity to the RH strain and they belong to the same clade. Isoptate KTG8 had 20% sequence dissimilarity with the reference, appearing to be an atypical strain and probably the most recent common ancestor of all the other isolates.

**Conclusion:** Although the majority of the isolates were recombinants of the reference RH strain, one was an atypical strain. Since RH strains are highly virulent, this presents dire consequences and/or often fatality in cases of congenital toxoplasmosis.

**Keywords:** Clonal lineage, atypical strains, RH strains, pregnant women, toxoplasmosis, Nigeria, Mali

322E Changes in the expression of glucose transporters before and after evagination of the scolex in *Taenia solium* David Castañeda Carpio1, Juan Blume La Torre1, Renzo Gutierrez-Loli1, Cristina Guerra-Giraldez1 1) Laboratorio de Proliferación Celular y Regeneración, Facultad de Ciencias y Filosofía. Universidad Peruana Cayetano Heredia. Lima, Perú.

*Taenia solium* develops into a sexually mature flatworm exclusively in the human gut; its eggs are excreted with the host’s feces, contaminating water, soil, and food. The evagination of the scolex from the ingested larval cyst and the consequent cellular proliferation are critical processes for the parasite’s development and growth into the adult form.

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Changes in the metabolism and glucose transport in proliferating tissues and during regeneration have been reported for other flatworm species. In *T. solium*, glucose transporters TGTPI and TGTTP2 have been identified, as well as a hypothetical SGLT1-like sodium-glucose cotransporter in an aberrant, hyperproliferative larval form. However, it is not known if these transporters play a significant role in the parasite’s metamorphosis.

We found variations at the mRNA level of glucose transporters using RT-qPCR on *T. solium* cysts obtained from pig muscle and incubated with taurocholic acid (TA), a bile component that induces evagination. First, we identified the sequence for a *T. solium* SGLT1-like transporter homologous to transporters of the MFS superfamily from different species of flatworms. This transporter showed higher mRNA levels 24h and 120h after the incubation with TA, compared with cysts incubated without the inducer. The mRNA levels of TGTPI also had a slight increase after incubation with TA. Furthermore, we identified a sequence for another SGLT-like transporter in *T. solium*, homologous to transporters of the mammalian SLC5-6 superfamily.

This work is a first step in understanding how glucose metabolism is involved in *T. solium* development. We propose to evaluate the expression of proteins related to the glycolytic pathway and the TCA cycle. Finally, the study of signaling pathways associated with metabolic regulation would provide more information about the nutritional requirements during parasite development.

332E Characterization of a novel endolysosomal complex localized Ca²⁺ binding protein during the *Toxoplasma gondii* lytic cycle

Noopur Dave1, Gustavo Arribazabalaga1 1) Indiana University School of Medicine.

In order to complete the lytic cycle, *Toxoplasma gondii* must sense and adapt to ion fluctuations as it moves from intracellular and extracellular environments, and through different tissue types within the human host. In particular, calcium transients within the parasite play a crucial role during motility, invasion, and egress. While progress has been made in identifying the calcium dependent signaling pathways that regulate the lytic cycle, the proteins and mechanism that directly regulate calcium homeostasis and fluxes are largely unknown. To address this knowledge gap, we have been identifying and characterizing proteins that contain Ca²⁺-binding properties and Ca²⁺ channels and pumps. The uncharacterized protein TgGT1_255660, which we have named TgEFP1, is predicted to have two calcium binding EF-hand domains located at the C-terminal end of the protein, along with an N-terminal signal peptide. To determine its localization, we endogenously tagged TgEFP1 with a triple hemagglutinin (HA) epitope tag. Immunofluorescence assays of tagged cell lines show that TgEFP1 co-localizes with the plant-like vacuole marker, TgNH3E, and is abutting to the Golgi as detected with TgSortilin. In non-dividing intracellular parasites, TgEFP1 is concentrated at the plant-like vacuole and Golgi compartments. During early division states, TgEFP1 staining appears diffuse as the daughter parasites start to form compartments of each daughter parasite. Interestingly, we also observed TgEFP1 accumulating within the parasitophorous vacuole (PV) in both non-dividing and dividing parasites. CRISPR/Cas9 strategies were used to generate TgEFP1 knockout (EFP1 KO) cell lines. Phenotypic analysis of EFP1 KO parasites, including ionophore induced egress assays, are being conducted to understand the role of TgEFP1 in Ca²⁺ signaling during the lytic cycle of the parasite. Localization of TgEFP1 to the plant-like vacuole and Golgi compartments, along with the PV, suggest a novel role of the endolysosomal system and the PV in *Toxoplasma* Ca²⁺ homeostasis.

332E Host adaptation in *Toxoplasma gondii*- What makes this parasite the most successful eukaryotic parasite in nature? Adit Noor1, John Boothroyd1 1) Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California, USA.

Toxoplasmosis is a life-threatening opportunistic infection caused by the intracellular protozoan *Toxoplasma gondii*. *Toxoplasma* infects an estimated 30% of the human population. While the infection it produces in an otherwise healthy adult is in most cases asymptomatic, serious, even fatal disease can sometimes occur. *Toxoplasma* is considered one of the most successful parasites in nature based on its worldwide distribution, high prevalence and extremely broad host range (almost any cell type in nearly all warm blooded animals can be infected). We know that *Toxoplasma’s* virulence is highly dependent upon allelic variation, driven most likely by host environment acting as a selection force.

To gain insight into how *Toxoplasma* evolved to be such a great generalist, and to the effect the host has on the parasite’s virulence, gene expression and growth, we used an in-lab evolution study to look at adaptation to a host. This in lab evolution study involved serially passing *Toxoplasma* in tissue culture, in three different hosts: human, mouse and cow fibroblasts. Following approximately 1000 generations in these different host cell lines we assessed the growth, virulence and expression profile of the three adapted lines. Remarkably, parasites grown on cow cells show an increased ability to attach to the host cell by as much as 15-fold compared to those grown on mouse or human cells. We were able to identify two possible mechanisms for this phenotype: These cow adapted parasites show higher expression levels of particular parasite surface proteins involved in attachment, and a higher level of micronemal protein secretion. We preformed expression studies that resulted in identification of host-specific gene expression, and are testing their essentiality in different hosts. Additionally, we investigated the gene expression for their stability, by presenting the adapted-paraites with a stress and examining whether the parasites convert to a similar gene expression pattern regardless of passage history. We have also tested the virulence of these adapted lines, and show, for the first time, that passage history of the parasite affects the virulence in a specific host.

332E Mild starvation of *P. falciparum* increases tolerance to DHA

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Under stress, many eukaryotes activate a general response to ensure survival. Mild stressors may alter metabolism in a manner that enhances the survival of a subsequent, distinct stressor leading to a phenomenon termed “cross-tolerance.” We hypothesize that the stressing of *P. falciparum* through nutrient limitation leads to cross-tolerance. We specifically demonstrate that short-term growth under mild nutrient stress reproducibly leads to superior recovery from dihydroartemisinin (DHA) treatment. In these experiments, parasites are “primed” using hypoxanthine restriction (48-72h, 0.5μM hypoxanthine) or thiamine deprivation (36h, no thiamine) prior to application of DHA (6h, 200μM) under normal nutrient conditions. As expected, all DHA-treated samples demonstrate a lack of measurable growth for approximately 1 week, followed by progressively increasing proportions of mitochondrial membrane potential-positive parasites and increasing growth rates. Following this initial period, primed parasites recover faster and show higher cumulative parasite proliferation. For example, at 10 days post-DHA treatment, hypoxanthine- or thiamine-deprived samples display 4- or 9-fold greater cumulative proliferation, respectively, compared to non-primed controls. Evidence of cross-tolerance between a potent antimalarial and a distinct metabolic pathway has implications for studying the development of drug resistance, as well as general parasite biology. We are currently investigating whether cross-tolerance: 1) is applicable to survival of other drug resistance pathways is dependent on known *P. falciparum* stress response pathways. Furthermore, we presume that stress from alterations in nutrient levels, temperature, or gas composition occur more frequently in vivo environment; therefore, our priming-based scheme may better represent parasite behavior during drug administration in the human host.

332E Characterization of an Endoplasmic Reticulum-Resident Calcium-binding protein in *Toxoplasma gondii*

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Calcium signaling is universal and impacts almost every aspect of cellular life. In *T. gondii*, Ca²⁺ signaling stimulates specific features of the parasite infection cycle and a number of known Ca²⁺ signaling elements are important for its parasitic cycle. Initiation of motility and the subsequent invasion and egress events have been shown to be activated by the release of intracellular Ca²⁺ stores. The endoplasmic reticulum (ER), likely the main Ca²⁺ store in the cell is
important for both Ca2+ homeostasis and signaling. We identified a calcium binding protein (Tg229480), an orthologue of the Plasmodium ER Calcium Binding Protein, or TgERC that localized to the T. gondii ER. Deletion of the TgERC gene resulted in mutants with reduced capacity to store Ca2+ in the ER. Using chemical Ca2+ indicators showed that when exposed to high extracellular Ca2+ ([Ca2+]~1.8 mM) the ER of the mutant parasites is unable to retain Ca2+. Phenotypic analysis of DTgerc showed slow growth. Further analysis with mutants expressing GCAmpP6 showed a delayed egress upon saponin addition compared to wild type, and the typical spike of Ca2+ that precedes egress was no longer and constant. Overall, TgERC play an important role in Ca2+ storage that lead to lytic cycle defects. Further analysis of TgERC partners at the ER and the effectors of this intracellular store Ca2+ release is ongoing

328E A PIRC44-regulated protein plays a role in cell-cycle progression of P. falciparum Marta Maia Machado1,2, Marie Jalovecká3, Severina Klaus1, Markus Gómez-Chávez 1) Center for Infectious Diseases, Heidelberg University Hospital, Heidelberg, Germany; 2) Graduate Program in Areas of Basic and Applied Biology, Instituto de Ciencias Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; 3) Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, Ceske Budejovice, Czech Republic.

Plasmodium falciparum (P. falciparum) displays a remarkably different mode of reproduction and multiples through schizogony, where successive rounds of DNA replication and nuclear division form a multinucleated cell prior to cytokinesis. Strikingly, DNA replication and nuclear division occurs asynchronously although the nuclei reside in a common cytoplasm.

Molecular insight in the regulation of DNA replication came from a loss-of-function screen of schizont-stage kinases, which identified P. falciparum CRK4 as a major S-phase regulator. Phosphoproteomic profiling of P. falciparum CRK4 identified over 200 proteins that are likely regulated by this kinase. However, the molecular function of many P. falciparum CRK4-regulated proteins remains unknown.

Using inducible gene depletion and immunofluorescence imaging, we describe a novel molecular player, PF3D7_1440100, involved in P. falciparum cell-cycle progression. Initially described as conserved protein of unknown function, PF3D7_1440100 was recently annotated as putative cohesin complex subunit. Bioinformatic analysis suggested that it contains a Rad21/Rec8 domain, indicating that it is involved in sister chromatid cohesion during mitosis and meiosis. To investigate the molecular function in P. falciparum, we added a triple HA-tag as well as the glmS-ribozyme to PF3D7_1440100. The addition of an enzymatically-inactive version of the ribozyme had no effect on the parasite, regardless of presence or absence of the inducer. When the active ribozyme was tagged to PF3D7_1440100, parasites showed an approx. 20% reduction in growth after 3 cycles, even in absence of the inducer, potentially due to leakiness of the system. Addition of the inducer further reduced parasite growth by approx. 20%. Immunofluorescent staining showed that PF3D7_1440100 localizes to the periphery of the nucleus, forming a dotted or ring-shaped structure. Co-staining with an antibody detecting CenP, a variant histone that marks the centromere, suggests that PF3D7_1440100 has a centromeric localization. In addition, co-staining with an anti-centromere antibody shows that PF3D7_1440100 is in close proximity to the centriolar plaque. Together our data describes an additional protein involved in cell-cycle progression and suggest that P. falciparum CRK4 may also be involved in the regulation of chromosome segregation.

329E Inhibition of Poly(ADP-Ribose)Glycohydrolase activity affects lysosomal function and hampers T. cruzi invasion in Vero cells Salome C Vilchez Larrea1,2, Silvia H Fernandez Villamil1,2 1) Instituto de Investigaciones en Ingeniería Genética y Biología Molecular “Dr. Héctor N. Torres”; 2) Universidad de Buenos Aires.

Chagas disease is a potentially life-threatening protozoan infection but, despite its high incidence and large economic costs associated to it, effective pharmacological treatments are lacking. The search for new anti-chagas drugs has focused on potential targets in the parasite itself but heterogeneity among different strains of Trypanosoma cruzi - the etiological agent- has hampered these efforts. Like other protosozoa, T. cruzi invades the host cell and this complex interplay can determine the outcome of the infection: the parasite must manipulate host cell signaling pathways to achieve its purpose. Therefore, targeting the host signals that promote T. cruzi infection can be therapeutically valuable. Poly(ADP-ribose) (PAR) – crucial for DNA damage response among other processes-participates in host cell response to the parasitic infection: Poly(ADP-ribose)polymerase-1 inhibitors decrease T. cruzi infection while Poly(ADP-ribose)glycohydrolase (PARG) inhibition or silencing almost completely abrogates it, raising interest in PAR signaling and the role of PARG in the host-parasite interaction.

PAR levels raised early after infection (15 min) and remained elevated during the complete cell infection cycle, as determined by ELISA using a PAR-detecting reagent. PARG inhibition by DEA 1 μM or silencing by shRNA caused reduced T. cruzi cell invasion, indicating that PARG might be important during this initial step. T. cruzi can invade the host cell by lysosome-independent, lysosome-dependent and autophagic pathways but they must all culminate in the fusion of the trypomastigote-bearing parasitophorous vacuole (TCpv) to lysosomes. Absence of PAR activity did not hamper the formation of TCpv with early endosomal characteristics as shown by staining against EEA1 (early endosomal antigen) or the use of a FYVE-eGFP probe to detect PIP3-rich vacuoles, nor did it affect infection levels when cells were subjected to nutritional stress, suggesting PARG is not participating in the initial stages of the lysosome-independent and autophagic pathways. However, PARG activity seems crucial for lysosomal function: PARG-inhibited or silenced Vero cells showed reduced DQ-BSA Red and Lysotracker DND-99 staining, indicating proteolytic activity and pH are altered. A drastic reduction in LAMP-1 signal was also detected. PARG inhibition and silencing also appears to affect the reorganization of host cell cytoskeleton during T. cruzi invasion. These results indicate that PARG activity is important for the maintenance of lysosomal activity, which is crucial for the initial steps of T. cruzi infection.

330E Placental immune response against Toxoplasma gondii could promote fetal maintenance but congenital transmission in late pregnancy Fernando Gómez-Chávez1,2, Dolores Correa1 1) Laboratorio de Inmunología Experimental, Instituto Nacional de Pediatría, Secretaría de Salud, México; 2) Cátedras CONACYT, México.

Congenital toxoplasmosis is caused by Toxoplasma gondii vertical transmission from mother to offspring during pregnancy. It may cause neuro-ophthalmic or even systemic problems to the fetus, which may last for life. The immunobiology subjacent to vertical transmission has not been elucidated, although in vitro and in vivo experiments of our work and others, suggest that several receptors of the immune response -supposedly related to immune protection- may paradoxically favor congenital transmission. The FcRn mediates IgG transport from mother to fetus through the placenta, so it is one of the main passive immunity transfer phenomena, but it could also get the parasite closer to the fetal face at the placenta. Cytokines like IL-1β, and TGF-β are involved in a finely regulated response which if properly balanced, let to tachyzoite- replication control and “healthy” inflammation regulation, but their role at the placenta has not been thoroughly studied in congenital toxoplasmosis.

In the present work, we intended to determine whether T. gondii infection can modulate the expression of FcRn, Gal-3, IL-1β, and TGF-β in the mouse placenta in a model of congenital infection. Pregnant Balb/c females were infected i.v. with 1x105 tachyzoites of the ME49 strain or vehicle, at day 15.5 post coitum. Placental infection was verified by real-time PCR. Besides, the FcRn, Gal-3, cytokines IL-1β, and TGF-β mRNA expression was tested 24 hours later by qRT-PCR.

We found that infection relates to higher expression of the FcRn in the placenta. This may partially explain increased percentage of transmission when mothers are infected at the end of pregnancy. We also found an induced expression of TGF-β, related to lower transmission rate and milder
explain third trimester congenital infections described phenomena in humans.

Malaria remains a worldwide concern with over 200 million cases and just below half a million deaths per year. Resistance to artemisinin-based therapies is also on the rise and has been linked to a decreased rate of the parasite's hemoglobin endocytosis and digestion pathway. The lizard malaria parasites Plasmodium flindoriscante, P. azuruophilum, and P. leucocytoea exhibit a unique niche partitioning within the same vertebrate host: Plasmodium flindoriscante inhabits red blood cells and produces hemozoin as a byproduct of hemozoin digestion. P. azuruophilum also inhabits red blood cells but does not appear to produce hemozoin, and P. leucocytoea inhabits white blood cells and, therefore, survives without hemozoin digestion. Elucidating how these lizard malaria parasites survive without hemozoin digestion will provide useful information that could be applied to resistant human infective malaria parasites. Using a comparative transcriptomics approach, we have found that key enzymes involved in the hemozoin digestion process have been retained in the genome and are transcribed by all three lizard malaria species.

332F Assessment of known Molecular Markers of Resistance Markers to Sulphadoxine-Pyrimethamine in the Buea Health Area SouthWest, Cameroon Ntui Vincent Ntui-Njock1, Tobias Apinjoh2 1) University of Buea, Southwest Cameroon; 2) University of Buea, Southwest, Cameroon.

Malaria remains an enormous public health concern especially in sub-Saharan African children, pregnant women and immuno-compromised adults. This has been attributed partly to the rapid and wide spread nature of anti-malaria resistant parasite strains that are compromising chemotherapy and chemoprevention. Although mutations in genes associated with anti-malaria resistance have been widely documented worldwide, this information is limited in Cameroon. This study sought to assess the curent status of Sulphadoxine-Pyrimethamine (SP) by genotyping Single Nucleotide polymorphism (SNP) in Pfdhps and Pfdhr genes with implication for IPTp-SP in the Buea Health Area Southwest Cameroun. Venous blood was collected by Venipuncture and malaria parasitemia determined by light microscopy. Parasite DNA was extracted by positive samples by Chelex-PBS method. SNP genotyping was then undertaken by nested PCR followed by Alleles Specific Restriction Analysis (ASRA) and a confirmatory deep sequencing. A total of 647 individuals, aged 0.6 to 80 years were enrolled, 53.5% of which were infected with the malaria parasite. Age(p<0.001), gender (p<0.001), febrile status (p<0.001), altitude of residence (p<0.001) and Pfdhfr genes with implication for IPTp-SP in the Buea Health Area.

333F Exploring growth inhibition and invasion blocking activity of different low molecular weight heparin against Plasmodium falciparum parasite Muqdad Hmoud1,2, Paul Horrocks1 1) Keele University, UK; 2) American University of Iraq Sulaimani, Iraq.

Background: Despite the global effort to decrease mortality and morbidity, malaria still causes 435,000 deaths and more than 200 million infection cases according to World Health Organization report 2018. Plasmodium falciparum is the most lethal malaria parasite among other malaria spp, and account the majority of malaria death cases. Antimalarial drug resistance has emerged in many affected areas, representing the hallmark malaria treatment treatment delay and failure. P. falciparum drug resistance has been reported to all known antimalarial therapies. There is high demand to develop a new approach to face antimalarial drug challenge. Heparin has anti-plasmodial activity by inhibiting P. falciparum growth via blocking merozoite invasion into erythrocyte. This activity seems to be mediated by interfering with major merozoite surface (MSPs), resulting in merozoite failure to invade the erythrocyte and establish a new life cycle. Heparin has been used clinically to treat patients with malaria infection, but due to its highly anticoagulation activity, heparin has been halted to be used as an adjunct therapy. Whilst heparin and other heparin mimetics their growth inhibition activity has been evaluated, a systematic exploration of this activity of range of Low Molecular Weight Heparins (LMWHs) has not. In this study, a systematic side by side comparison of the in vitro growth inhibition of commercial LMWHs are explored. In addition, exploration of using LMWHs as adjunct therapy has been evaluated.

334F Eukaryotic Pathogen, Vector and Host Omics Data-Mining for Everyone Brian Brun1, Omar Harb1, Mark Hickman1, Jessica Kissinger2, David Roos1, Susanne Warrenfeltz2 1) University of Pennsylvania; 2) University of Pennsylvania.

VEuPathDB provides free online access to genomic-scale data-mining resources for >400 species of eukaryotic pathogens and related taxa, including many protozoan parasites of interest to MPM on disease – as well as arthropod vectors of disease, and selected host species. This knowledgebase empowers biologist end-users to leverage diverse multi-Omics datasets, without requiring specialized analytical or computational skills. Advanced search capabilities, data visualizations and analysis tools are applied to genomic-scale data, enabling the development & testing of in silico hypotheses, and facilitating the discovery of meaningful biological relationships. Available information includes genome sequence & population-level variation data; manually-curated & automatically generated annotation (including thousands of User Comments from the community); epigenetic, transcriptomic & proteomic data (multiple analytical platforms); pathway information, including metabolomic datasets; genome-wide phenotypic analyses (knock-out/knock-down phenotypes, subcellular localization, etc), information on host-pathogen interactions, and more. VEuPathDB applies standardized bioinformatics workflows and analyses, providing a consistent view of complementary datasets for diverse strains and species, from multiple researchers, using a phylogenetic framework to facilitate cross-species functional inference via orthology. Data-mining may involve simply browsing integrated gene/pathway records; visualizing diverse datatypes aligned within the genome browser; sophisticated Search Strategies to identify genes or features that share biological characteristics; a user-friendly Galaxy workspace for analysis of private user data in the context of public datasets; and the MapVEu tool for visualizing, searching, analyzing and downloading geographically-mapped genomic, phenotypic and data. The user support team provides email help, social media posts, video tutorials, webinars, workshops, and more. (A virtual) help desk will be continuously staffed during all poster sessions at MPM, or email help@VEuPathDB.org for more information. VEuPathDB is one of two NIAID-supported Bioinformatics Resource Centers, with additional support from the Wellcome Trust, the Gates Foundation, and others.

335F Toxoplasma gondii matrix cyst wall protein MAG1 is a secreted immunomodulatory effector and critical for acute virulence and establishing chronic infection Tadakimi Tomita1, Debanjan Mukhopadhyay2, Jeroen Saeij2, Louis Weiss1 1) Albert Einstein College of Medicine; 2) University of California, Davis.

Our initial effort to identify novel cyst wall proteins serendipitously led us to the re-discovery of a quarter-century-old cyst wall and vacuolar matrix protein MAG1 as a novel secreted immunomodulatory effector. MAG1 is a dense granular protein abundantly present in parasitophorous vacuolar
matrix in tachyzoites and cyst wall in bradyzoites. Our previous inteरсοmе analysis identified MAG1 as a hub protein in the cyst wall composition. In our current study, we have demonstrated that MAG1 is secreted and can function as a virulence factor. MAG1 parasites produced in vivo in macrophages showed reduced virulence and dissemination. The chronic stage, Δmag1 parasites increased almost no brain cysts. Therefore, MAG1 is critical for acute virulence and chronic cyst establishment. To identify the mechanism of attenuated patоlogy of Δmag1 parasites in mouse infection, various immune responses were screened in vitro using bone marrow derived macrophages (BMDM). The infection of BMDM with Δmag1 parasites induced a significant increase in IL-1β secretion, which is a hallmark of inflammasome activation. Trans-complementation of MAG1 in the BMDM cells prevented this Δmag1 parasite-induced IL-1β release indicating that secreted MAG1 in host cytosol dampens the inflammasome activation. Further deleting a known inducer of IL-1β release GRA15 in Δmag1 parasite completely inhibited the IL-1β release. This suggests that MAG1 has a role as an immunomodulatory molecule and by suppressing inflammasome activation it would favor survival of this parasite and the establishment of latent infection.

Characterization of genes involved in Glycosylation process as potential virulence factors in *Leishmania braziliensis* Gabrielle Ariadine Bento1, Mariana Santos Cardoso1, Lucas Lorenzon2, Viviane Grazier-Silva3, Marcos Vinicius Santos Borges1, Tiago Antônio de Oliveira Mendes4, Ricardo Toshio Fujiwara1, Ângela Kaysel Cruz2, Daniella Castanheira Bartholomeu1 1) Department of Parasitology, Federal University of Minas Gerais; 2) Ribeirão Preto School of Medicine, University of São Paulo; 3) Department of Biochemistry and Immunology, Federal University of Minas Gerais; 4) Department of Biochemistry and Molecular Biology, Federal University of Viçosa.

Leishmaniasis presents a wide variety of clinical manifestations depending on the *Leishmania* species that causes the disease and the host immune response. However, the understanding of the species-specific adaptations responsible for parasite virulence and tropism is still limited. Comparative genomic analysis among *Leishmania* species, previously performed by our group, revealed that some genes involved in glycocalyx biosynthesis are absent in *L. tarentolae*, a non-infective species for mammals. Flow cytometric analysis corroborated these *in silico* findings. Thus, the aim of this study was to perform the functional characterization of two genes associated with the glycosylation process in *L. braziliensis*. Among the species evaluated, the first gene is absent only in *L. tarentolae*, whereas the second gene is absent in *L. braziliensis* and *L. tarentolae*. We quantified the mRNA expression levels of these genes by RT-qPCR in promastigotes, and axenic and intracellular amastigotes of *L. braziliensis*, *L. donovani*, *L. infantum* and *L. tarentolae*. The first gene presented a higher expression level in the intracellular amastigotes in the pathogenic species and, as expected, no expression was detected in *L. tarentolae*. For the second gene, the results confirmed the absence of expression in *L. braziliensis* and *L. tarentolae*, and revealed higher expression levels in amastigotes of the viscerotropic species. Additionally, we were able to generate *L. braziliensis* mutants by deleting the first gene, using CRISPR technique, and by expression of the second gene. The mutant parasites were identified by conventional PCR and by RT-qPCR. Analysis of gene expression by western blot and evaluation of phenotypic changes through *in vitro* and *in vivo* infections are underway. We hope that this work can contribute to a better understanding of the mechanisms of infection of *Leishmania* and these enzymes can be good candidates for therapeutic targets.

Keywords: *Leishmania*, glycosylation, gene knockout, mutant parasites, virulence factors

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The metamorphosis of *Taenia solium* into its adult form depends on Notch signaling Juan Blume1, David Castañeda1, Renzo Gutierrez1, Cristina Guerra1 1) Laboratorio de Proliferación celular y regeneración. Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru.

*Taenia solium* is a cestode with two distinct stages: the adult, an intestinal flatworm, and the larval cyst that can lodge in muscles or brain tissue of humans and pigs that ingest the parasite’s eggs. The worm stage can grow up to 3 meters by forming proglottids that resemble the segments present across various species. Since genes from the Notch pathway play key roles regulating animal body patterning and segmentation, we tested if this signaling pathway participated in the evagination of the scolex of *T. solium*. This is a critical process for the metamorphosis of the larva into the adult worm, as it enables the parasite to adhere to the human gut lining, perhaps even triggering the formation of proglottids. For this aim, groups of cysts were collected from infected pig muscle and then cultured in the presence of taurocholic acid (TA), to induce evagination, or DAPT, a γ-secretase inhibitor, to inhibit Notch signaling.

After five days, 92% of the cysts treated with TA had evaginated. The induced juvenile worms showed increased expression of Notch1 and the Notch ligand Delta4, measured by RT-qPCR, compared with the untreated controls and the inhibited cysts. Conversely, 93% of the cysts treated with DAPT for five days failed to evaginate the scolex. Compared with the untreated controls, the mRNA level of Notch1 and Delta4 decreased in the presence of the inhibitor, while the expression of the Notch target gene Hes1 increased. These results show that Notch genes are necessary for evagination of the scolex. The study of this pathway in *T. solium* could prove useful in the search for targets to inhibit the development of the parasite into its sexually mature stage in the human gut and thus prevent dissemination.

Murine gut parasite *Trithromomonas musculus* drives changes in the metabolism of intestinal microbiota Ana Popovic1,2, Eric Cao2, Michael Grigg3, Arthur Mortha2, John Parkinson1,2 1) The Hospital for Sick Children, Toronto, ON; 2) University of Toronto, Toronto, ON; 3) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

Eukaryotic microbiota, including protozoa, helminths and fungi, constitute important members of the intestinal microbiome. Among the protists, well known pathogens such as *Cryptosporidium* and *Entamoeba histolytica* have been shown to induce marked changes in the host immune system as well as resident bacterial populations. By contrast, relatively little is known about the impact of commensal or pathobiont species on the gut environment, despite their abundance. In µMT mice, there was a concurrent decrease in *Akkermansia* abundance. It total, 35 bacterial taxa significantly changed in mice infected with *Trithromomonas musculus* (Tmu) was previously shown to induce the host inflammasome, in an otherwise asymptomatic infection, protecting mice from subsequent *Salmonella* invasion.

Here, we attempt to reveal the relationship between Tmu colonization and the gut bacterial composition and metabolism over a four-week infection course in wildtype and B-cell deficient (µMT) C57BL/6 mice, using 16S and metatranscriptomic sequencing. We find that Tmu incurred significant diversification of the microbiome in both host genotypes, beginning one week post infection. We noted expansions in Bacteroidetes and Firmicutes taxa, as well as an increase in *Bifidobacterium* abundance. In µMT mice, there was a concurrent decrease in *Akermansia* abundance. It total, 35 bacterial taxa significantly changed in abundance over time, five exhibiting host-genotype-specific changes. Preliminary analysis of caecal microbial RNA data suggests distinct differences in both bacterial and Tmu gene expression between early and late stages of infection (two and 28 days, respectively), with an increase in Tmu carbohydrate metabolism.

Single cell RNA sequencing of 6000 Tmu parasites, isolated from the caeca of two additional C57BL/6 mice, allowed us to identify 15 distinct Tmu subpopulations based on gene expression. We designed fluorescent RNA-binding probes to target marker genes uniquely expressed in each subpopulation. Using a fluorescence-activated cell sorting assay, we applied these probes to parasites isolated from active infections, and verified the presence of four subpopulations to date.
Further characterization of microbial metabolic changes during Tmu infection will reveal the interplay between this organism, and potentially its human counterparts, the bacterial microbiota and the host.  

339F Malaria parasites require a divergent heme oxygenase for apicoplast transcription and biogenesis Amanda Mixon1, Sebastian Nasamu2,3, Jose Caveiro4, Celine Slam1, Jacquin Niles2, Daniel Goldberg3, Paul Sigala1 1) University of Utah; 2) Massachusetts Institute of Technology; 3) Washington University; 4) University of Tokyo.  

Plasmodium malaria parasites are divergent eukaryotic cells that have evolved numerous metabolic adaptations to survive and proliferate within human and mosquito host environments. During infection of human red blood cells, parasite import and digest vast amounts of hemoglobin. This process releases a large amount of free heme, which parasites predominantly sequester as crystalline hemozoin. Nevertheless, P. falciparum retains a divergent heme oxygenase (PHO, PF3D7_1011900) that lacks the catalytic His residue and has lost the ability to degrade heme. We have localized PHO to the parasite apicoplast, which may suggest evolutionary acquisition from the cyanobacterial ancestor of this organelle based on structural and low-level sequence homology of PHO to cyanobacterial HO. Conditional knockdown of PHO is lethal to parasites, is rescued by isopentenyl pyrophosphate (a critical apicoplast-produced metabolite), and blocks apicoplast biogenesis. PHO has signal and transit peptides that confer apicoplast targeting but, in contrast to other apicoplast-targeted proteins, PHO retains most of its positively-charged transit peptide upon plastid import. Episomal rescue experiments indicate that these N-terminal residues are critical for the essential function of PHO. By immunoprecipitation, we have discovered that PHO selectively associates with apicoplast DNA and interacts with multiple apicoplast gyrase and helicases. Upon PHO knockdown, parasites fail to increase apicoplast-encoded RNA levels by RT-qPCR but appear to replicate their DNA normally, suggesting a critical role for PHO in transcription of the apicoplast genome. We hypothesize that PHO has been functionally repurposed to facilitate the early steps of DNA unwinding required for apicoplast transcription. PHO provides an example of the divergent molecular functions evolved by Apicomplexan pathogens during adaptation to intracellular parasitism and which can serve as new parasite-specific targets for therapeutic intervention.  

340F In silico analysis of ADMET properties for compounds that present anti-schistosoma activity Sandra Gava1, Naira Tavares1, Izabella Batista1, Tom Armstrong2, Bernardo Moreira3, Franco Falcone3, Guilherme Oliveira4, Marina Mouraâ1, 1) Renê Rachou Institute – FIOCRUZ/Minas, Belo Horizonte, Brazil; 2) University of Nottingham, Nottingham, United Kingdom; 3) Liebig-Universität Gießen, Gießen, Germany; 4) Yale Institute of Technology, Belém, Brazil.  

The in silico prediction of pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) are reasonable indications to guide the in vivo screening of new drug candidates. Recently, our group did a docking-based virtual screening that enables the identification of potential Protein Kinases inhibitors. These compounds were prioritized and screened in vitro against the parasite Schistosoma mansoni. Compounds that promoted mortality or phenotypic changes were considered active. Here, we present the predicted ADMET properties for active compounds, using the pkCSM web server. All the active compounds, except one, obeyed with Lipinski’s rule of five, such as logP<5, MW<500 Da, HBA<10, and HBD<5. The predicted solubility in water at 25 ºC ranged from 2.8x10-7 to 3.1x10-3 mol/L. The estimated values for human intestinal absorption ranged from 100 to 60.7%, denoting they are highly absorbed. Also, the membrane permeability predicted for the Caco-2 cells ranged from 1.7 to 0.01 (logPapp in 10-6 cm/s), indicating that they are easy to absorb. The compounds had volumes of distribution (Vdss) ranging from -1.1 to 1.3, with higher Vdss values indicating that the compounds are more likely to be distributed in tissues rather than in plasma. Predicted values for blood-brain barrier (BBB) permeability (2.9 to 0.5) indicated that some of the active compounds readily cross the BBB (logBB>0.3) and some are poorly distributed to the brain (logBB<1). The predicted blood-brain permeability area product (logP70) (-0.3 to 1.5), indicated that some compounds could be able to penetrate the central nervous system (logP50~2) and some are unable to penetrate (logP50<3). Concerning the interaction with drug transportsers, 64.8% of the active compounds were predicted as a P-glycoprotein substrate, and 76.1 and 64.8% as an inhibitor of P-glycoprotein I and II, respectively. About the drug metabolism mediated by cytochrome P450 (CYP) enzymes, 2.3, 63.3, and 64.8% of the active compounds were predicted to be a substrate for the isozymes 2D6, 2C9, and 3A4, respectively. Regarding drug excretion, 16% of the active compounds were predicted to be excreted out from the human body. The majority of active compounds (92%) were predicted to be hepatotoxic, 1.1% were predicted as an hERG I inhibitor, and 81.8% predicted as an hERG II inhibitor. None of the active compounds were predicted to promote skin sensitization and only 9.5% of them presented AMES toxicity. Oral rat acute toxicity ranges from 1.6 to 3.2 mol/kg, and oral rat chronic toxicity ranges from 0.3 to 2.6 mol/kg/day. Together, these parameters allow us to prioritize candidates for further in vivo tests, seeking to explore the potential of these anti-schistosoma drugs in the mammalian host.  

341F Bioinformatic and cell-based tools for reverse and forward genetics using CRISPR in mosquitoes Raghuvar Viswanathan1, Enzo Mameli1,3, Jonathan Rodiger1, Tonya Colpitts3, Yanhui Hu1, Stephanie Mohr1, Norbert Perrimon1,2 1) Harvard Medical School; 2) Howard Hughes Medical Institute; 3) Washington University; 4) Columbia University; 5) University of Tokyo.  

Celine Slam1, Jacquin Niles2, Daniel Goldberg3, Paul Sigala1 1) University of Utah; 2) Massachusetts Institute of Technology; 3) Washington University; 4) University of Tokyo.  

Mosquito-borne diseases present a worldwide public health burden, yet tools for designing and conducting CRISPR experiments in mosquitos trail behind those of well-annotated genetic organisms such as Drosophila. Here, we present a bioinformatic toolkit for mosquito CRISPR reverse genetics and a platform for cell-based CRISPR knockout generation and pooled CRISPR screening in mosquitoes. First, we created an orthology-based search tool allowing resources described represent a powerful and open-source approach for reverse and forward genetics in mosquitos.  

342F Mechanistic Insight into the Invasion Apparatus for Infection by Microsporidia Parasites Breanne Budaitis1, Pattana Jaroenlak1, Manu Prakash2, Damian Ekiert1, Gira Bhabha1 1) Skirball Institute of Biomolecular Medicine, NYU, New York, NY; 2) Stanford University, Stanford, CA.  

Microsporidia are a highly divergent group of obligate intracellular parasites that infect a wide range of hosts including protists, invertebrates, and vertebrates. All microsporidia species have a specialized harpoon-like invasion apparatus called the polar tube (PT) to gain access to the host cell. The PT is coiled and packed like a spring in dormant spores. Upon activation, it reorients and extends the apical end of the spore as a linear tube providing a conduit for passage of infectious material to the host cell. Despite observation of the PT 100 years ago, little is understood about the mechanistic basis that underlies PT firing and transport of infectious material. One possibility is that the tube everts, reminiscent to a finger of a glove turning inside out. We used high-speed optical microscopy to capture PT firing in vitro and find that changes in viscosity do not affect the kinetics of firing, consistent with a model of eversion. Current efforts to understand the molecular and biophysical factors that power PT eversion are underway and whether this is a mechanism conserved across microsporidia.  

343F Knowledge, attitude and practices associated with toxoplasmosis in pet owners of province Punjab, Pakistan Abrar Hussain1, Sabir Hussain1, Ubaid -Ur-Rehman Zia1, Olivier Saparagoun2, Jeffery HO2 1) University of Veterinary and Animal Sciences; 2) City University Hong Kong.  

The present study aimed to evaluate the pet owners’ knowledge, attitude, and practices regarding toxoplasmosis in province Punjab, Pakistan. Only those pet owners were included in the study who had at least one cat as their pet. They were face to face interviewed for obtaining information subjected to their knowledge about toxoplasmosis and its zoonotic importance. Out of 200, there were 124(62%) respondents didn’t know about the toxoplasmosis While 76(38%) were familiar with toxoplasmosis, the majority of the 50(65%) out of those 76 didn’t know about its zoonotic nature. There were 82(41%) who usually
offer raw meat to their cats which is a major risk factor for cat getting these diseases because of the normal presence of Toxoplasma gondii in cow and goat meat. Out of 200, there were 138 (69%) who didn’t have a litter box for cats and 62 (31%) had the litter box. Out of 62 with litter box facility 50(80%) didn’t use gloves during disposing the litter from litter box which is the major risk factor for causing zoonotic toxoplasmosis. From the chi-square test, we deduce that graduation as an educational status was significantly associated with knowing the toxoplasmosis as a disease of the cat (P=0.003). We also deduce that females had a significant association with knowing about toxoplasmosis is a disease of the cat (P=0.024) even having the same educational status, because of having more elements of care for animals by nature. Our results showed that graduation as an educational status was also significantly associated with knowledge of these diseases being zoonotic (P=0.001). This knowledge of toxoplasmosis being zoonotic disease compelled them to adopt some preventive measures like using gloves during the cleaning of the litter box. The data also depicted the significant association between the offering of raw meat with appearing some symptoms of toxoplasmosis (P=0.037). The raw meat consumption is the risk factor not only for toxoplasmosis but many other diseases. The use of properly cooked meat or ready-made cat feed is also a preventive measure for toxoplasmosis.

344F Knowledge, Attitude and Practices of Echinococcus granulosis in different slaughter house worker of Punjab Pakistan SABIR HUSSAIN1, Abrar Hussain1, Olivier SPARAGANO2, Jeffery HO 2) University of Veterinary and Animal Sciences, Lahore; 2) City University of Hong Kong.

The present study aimed to evaluate the livestock workers’ Knowledge, attitude, and practices regarding Echinococcus granulosis in Pakistan, Punjab. A KAP survey was conducted among the livestock workers. They were face to face interviewed for obtaining information subjected to their knowledge about Echinococcus granulosis and its zoonotic importance. Among 50 different slaughterhouse workers, 45 (90 %) were unfamiliar about the zoonotic importance of Echinococcus granulosis and only 5 (10 %) recognized its zoonotic importance. Out of 50 slaughterhouse workers, 35 (70 %) did not know the association of canine kept nearby has a high potential of getting this disease. Among respondents 46 ( 84 %) of people that did not consider hydatid cyst as a potential source of infection. Out of 50 slaughterhouse workers, 27 (54 % ) did not use protective clothing and gloves which is a major risk factor for its zoonotic transmission.

On Chi-Square analysis, out of those workers whose education was significantly associated with knowledge of Echinococcus granulosis as compared to those having no formal education (P=0.004 ). On analyzing potential risk factors, Chi-square depicted that there was a significant association between zoonotic potential and education status. (P<0.002). There was a significant association of the hydatid cyst with the education level (P= 0.025). There should be a proper course that has to be passed by the workers before working in the slaughterhouses just not to protect themselves but also their families.

345F Genetic diversity of the neglected Plasmodium malariae parasite using targeted amplicon sequencing techniques Eniyoo Cheryll Oriero1, Deus Ishengoma1, Lucas Amenga-Etego3, Tobias Apinjob4, Soulama Issiaka5, Umberto D'Alessandro1, Abdoulayev Djimde6, Martin Meremikwu7, Alfred Amambua-Ngwa1 1) Medical Research Council Unit The Gambia at LSHTM, Fajara, Gambia; 2) National Institute for Medical Research, Tanga, Tanzania, United Republic of; 3) University of Ghana, WACCBIP, Legon, Ghana; 4) Faculty of Health Science, University of Buea, Buea, Cameroon; 5) Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; 6) Malaria Research and Training Center, University of Science, Techniques and Technology, Bamako, Mali; 7) University of Calabar Teaching Hospital, Calabar, Nigeria.

Plasmodium malariae is considered a benign parasite species, despite being the major co-infecting malaria parasite species that infect humans in sub-Saharan Africa. Recent completion of its reference genome paves way for new genomic studies aimed at understanding the biology and transmission dynamics of the parasite species. We optimized targeted amplification of ten P. malariae orthologous genes associated with antimalarial drug resistance in P. falciparum and characterized samples from across sub-Saharan Africa using targeted amplicon sequencing techniques. Specific primers were designed to amplify PmAAT1, PmAP2mu, PmATP6, PmCYTB, PmDHFR, PmDHPS, PmKelch13, PmCRT and PmMDR1 genes. Generated amplicons for each sample were pooled per target for library generation and downstream sequencing on the Illumina MiSeq platform, according to manufacturer’s protocols (Illumina Inc, USA). Two targets with small amplicon sizes, <1000bp (PmCYTB = 585bp and PmCRT = 729bp) were also analyzed by Sanger sequencing on the SeqStudioä Genetic Analyzer system (Applied Biosystems). Preliminary results from the SeqStudioä Platform shows no diversity with the PmCYTB gene. Knowledge of the population structure and diversity of P. malariae across endemic regions will help in monitoring local and regional transmission patterns and improve implementation of intervention tools such as drugs and vaccines.

346F Identification of immunogenic non- Variant Surface Glycoprotein (VSGs) surface protein on wild Trypanosoma vivax Kwadwo O. Oworze1, Yaw Aniwhe1, Theresa Manful Gwira1 1) West African Center for Cell Biology of Infectious Pathogens, Department of Biochemistry Cell and Molecular Biology, University of Ghana, Ghana.

The main challenge to vaccine and diagnostic development for trypanosomes, the causative agent for animal African Trypanosomiasis, is their potent antigen variation of their surface glycoprotein (VSGs) that densely covers their entire cell surface. Studies have shown that Trypanosoma vivax, second to T. brucei in prevalence, expresses less dense VSGs. However, preliminary data have shown the presence of non-VSG surface proteins. In this study, we identified a highly expressed non-VSG surface protein on T. vivax and determined its immunogenicity using synthetic peptides.

Two herds of cattle from Addidome and Bolgatanga were selected for the study. Blood was collected at 8 weeks intervals for 4 timepoints. The infecting trypanosomes were PCR-typed targeting the trypanosome tubulin gene cluster. In silico analysis of Spliced Leader RNA-sequencing data identified TvYA86_0029740, a hypothetical protein, as a highly expressed T. vivax surface protein. Peptides (P1-11) of its extracellular domain were synthesized. The immunogenicity of the peptides was tested against sera from selected cattle using ELISA and compared with a known T. vivax immunogenic recombinant protein (TvYA86_0045500).

Our data confirmed T. b. brucei as the most prevalent followed by T. vivax and T. congolense. Evidence of mixed infection with two or more trypanosome species were seen. Antibody response to the selected protein was significantly higher in infected cattle than naive cattle. Although antibody response in animals from Bolgatanga was significantly higher than that of Addidome, there was no significant difference in antibody response to the peptides across the various timepoints. P-6 was identified to be the most immunogenic with seropositivity of 100% to 89.1% of the recombinant protein. The magnitude of antibody response was also higher (p<0.0001) for P-6, 1.181AU (0.9705-1.368) than the recombinant protein 0.7056AU (0.3344-1.079). This data identified a possible immunogenic peptide for vaccine or diagnostic purposes and provides insight on the prevalence of trypanosome infection.

347F “So different yet so much alike”: a new cryptic species Microcotyle isyebi (Monogenea, Platyhelminthes) from Boops boops (Teleostei) off the Algerian coast Chahtinez Bouguerche1, Kamila Gharbi1, Delphine Gey 2, Doria Boubaach2, Naouel Mekarma1, Jean-Lou Justine 3, Fatima Tazeroutli 1) Université des Sciences et de la Technologie Houari Boumediene; 2) Service de Systématique moléculaire, UMS CNRS, Muséum National d’Histoire Naturelle, Sorbonne Universités, Paris , France; 3) Institut Systématique Évolution Biodiversité (ISYEB), Muséum National d’Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France.

The monogenean Microcotyle erythrina is atypical because it has been recorded from several fish host species in the Mediterranean Sea and Atlantic Ocean, in contrast to many species which are considered strictly specific. This could indicate a true lack of specificity or that several cryptic species are involved. This study is a partial attempt to solve this problem. Specimens of a monogenean resembling M. erythrina were collected from bogue, Boops boops, caught off Algeria. A comparison with published descriptions and with museum specimens of M. erythrina did not yield any clear morphological difference. However,
sequences of cytochrome c oxidase subunit I (COI) differed by 16.3% from that of \textit{M. erythrin} (from GenBank, material collected from the type-host \textit{Pagellus erythrin}) indicating that the species was different. The species from \textit{B. boops} is therefore described here as \textit{Microcotyle isyebi} was a new species, and differential diagnoses with \textit{Microcotyle} species from the Mediterranean and from sparsids are provided. These results suggest that a molecular re-evaluation of other \textit{M. erythrin}-like specimens from various fish hosts could reveal the existence of additional parasite biodiversity.

348F Transcriptional activation of multiple clonally variant genes in \textit{Plasmodium falciparum} after passage through transmission stages and exposure to human host conditions

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\textit{Plasmodium} spp. have evolved alongside humans for millions of years and possess a huge adaptive capacity. Clonally variant genes (CVGs) are key in this plasticity, being involved in processes like antigenic variation, erythrocyte modification, cell permeability or sexual conversion. These genes present stochastic switches between active and silent transcriptional states, generating phenotypic variability transmissible by epigenetic mechanisms. Transcriptional analysis is fundamental for deciphering the function and regulation of CVGs, yet as in general transcriptional patterns differ significantly between \textit{in vitro} and \textit{in vivo} conditions it can be difficult to extrapolate results between these two settings. On the other hand, it has been found that passage through transmission stages produces an epigenetic reset of \textit{var} and \textit{clag3} CVGs, with parasites entering the asexual blood cycle with “fresh” and diverse expression patterns. In order to identify the transcriptional differences between culture and human infection conditions and determine if other CVGs are submitted to an epigenetic reset during transmission stages, we performed a time-course transcriptomic analysis of parasites collected from four malaria-naïve volunteers injected with sporozoites in a Controlled Human Malaria Infection (CHMI) study and the parental NF54 line. Parasites from volunteers showed significant expression changes only in parasites entering the asexual blood cycle, and a burst of silent \textit{var} genes, an exported protein and, most notably, \textit{gdpH2}, which was fully silenced in the parental line. Moreover, \textit{var}, type \textit{A rifin} and stever families also presented altered expression patterns. No major differences were found between volunteers, and whole genome sequencing uncovered no relevant genetic changes. Furthermore, ChiP-seq analysis confirmed the epigenetic nature of the observed transcriptional changes. The use of CHMI trial samples confirmed the study of malaria parasites exposed to human host conditions but without the great variability of natural infections. We have been able to further confirmed that CVG expression varies significantly between \textit{in vitro} and \textit{in vivo} conditions and that the transcriptional differences observed can be explained by an epigenetic reset during transmission stages and posterior selection of certain phenotypes in the human circulation. The generation of transcriptional variation prior to exposure to the human host is an example of a bet-hedging adaptive strategy. To better understand the functional consequences of these transcriptional variations, we will perform phenotypic comparison of the parental and human passaged parasites.

349F Control of VSG expression by a phosphoinositide-regulated telomeric expression site complex

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\textit{Trypanosoma brucei} evades the host antibody clearance by periodically switching their variant surface glycoprotein (VSG) coat. They express only one out of hundreds of VSG genes at a time from one of the 20 telomeric expression sites (ESs). They change VSGs by switching transcription between ESs or by VSG gene recombination. We showed that a nuclear phosphatidylinositol phosphate 5-phosphatase (PIPSPase) associates with repressor-activator protein 1 (RAP1) within a 0.9 MDa multiprotein complex, and PIPSPasen knockdown results in transcription of all ES VSG genes. Here, we show that PIPSPasen localizes in the nucleus of \textit{T. brucei} procyclic forms (PFs), and its knockdown also results in the expression of all ES VSG genes. Using nanopore RNA-seq, we show that bloodstream form (BF) cells that exclusively express PIPSPasen catalytic mutant (D360A/N362A), which inhibit PI(3,4,5)P3 dephosphorylation, also express silent VSG genes. Chromatin immunoprecipitation (ChIP) show that PIPSPasen and RAP1 interact with ES sequences, namely 70 bp and telomeric repeats. We generated recombinant RAP1 as well as RAP1 domains (N-terminal), Myb (central), and Myb-like (MybL, C-terminal). We show that RAP1 binds to PI(3,4,5)P3 via its N-terminal (RAP1) domain. On the other hand, gel shift assays show that Myb and MybL bind directly to synthetic 70 bp and telomeric repeats. Using microscale thermophoresis, we show that RAP1 associates with telomeric and 70 bp repeats with dissociation constants (Kd) of 140 nM and 2.5 μM, respectively. Notably, PI(3,4,5)P3 inhibits RAP1 association with telomeric repeats, but not with 70 bp repeats. The Kd of RAP1 with telomeric repeats increases from 140 nM to 11 μM in the presence of PI(3,4,5)P3. To investigate how PI(3,4,5)P3 regulates RAP1 association with ES sequences \textit{in vivo}, we performed ChIP analysis in cells that exclusively express catalytic mutant of RAP1. The mutant cells show significant loss of RAP1 association with telomeric repeats compared to wild type cells. We propose that PIPSPasen activity regulates RAP1 association with telomeric repeats and thus silencing of VSG genes. Loss of PIPSPasen activity results in PI(3,4,5)P3 accumulation, which binds to RAP1 via the RCT domain. The binding displaces RAP1 from telomeric repeats, which likely affect ES chromatin organization and results in VSG transcription. Hence, PIPSPasen is essential for the exclusive expression and the developmental silencing of VSG genes.

350F Complete arrest of \textit{Plasmodium falciparum} liver stage development in parasites lacking Multidrug resistance protein 2 (MRP2)-ABC-Binding Cassette transporter 2 (ABCC2)

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ATP binding cassette (ABC) transport proteins constitute a highly conserved superfamily of membrane transporters with broad substrate specificities that includes pharmaceuticals, rendering their study clinically relevant. Numerous ABC transporters are encoded in the \textit{P. falciparum} genome, we analyzed the role of a \textit{c}-type ABC transporter called multidrug resistance-protein-2 (MRP2-or-ABCC2). In \textit{PfABCC2}-mCherry C-terminal epitope-tagged parasites, the \textit{Pbabc2}— showed normal asexual blood stage-, gametocyte- and mosquito stage development, indicating a dispensable role of the gene in these stages of the parasite life cycle. The liver stage phenotype was then characterized for the first time in FRG-huHep mice that have a humanized liver. Infection of these mice with \textit{Pfabc2}— sporozoites, demonstrated impaired liver stage development beyond day 2 after infection. \textit{Pfabc2}— livers still persisted in the liver on day 6, however they were considerably smaller than their wildtype counterparts with reduced DNA replication and developmental arrest at the early schizont stage. FRG-huHep mice that were challenged with wildtype \textit{Pf} sporozoites and subsequently infused with human red blood cells (RBCs) on Day 7 showed consistent transition to blood stage as confirmed by both RT PCR and blood smears. In contrast, \textit{Pfabc2}— sporozoite-infected FRG huHep mice did not become blood stage patent. Our results demonstrate a crucial role of \textit{ABCC2} in growth of \textit{Pf} liver stages. This suggests that \textit{PfABCC2} is a new attractive gene locus for liver stage attenuation and that \textit{PfABCC2}— parasites constitute a novel candidate for an early to mid-liver-stage arresting genetically attenuated parasite (GAP) vaccine.

351F The generation of extra-chromosomal DNA amplicons in antimalarial resistant \textit{Plasmodium falciparum}

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Extra-chromosomal (ec) DNAs are genetic elements that exist separately from the genome. Since ecDNA can carry beneficial genes, they are a powerful adaptive mechanism in cancers and many pathogens. For the first time, we report ecDNA contributing to antimalarial resistance in \textit{Plasmodium falciparum}, the most virulent human malaria parasite. Using pulse field gel electrophoresis combined with PCR-based copy number analysis, we detected two ecDNA elements that differ in structure and migration. Treatment with structure-specific nucleases revealed that one ecDNA is likely linear fragments of another large, complex ecDNA molecule. Using deep sequencing, we show that ecDNA originates from the chromosome and expansion of an ecDNA-specific sequence may improve its segregation or expression. We speculate that ecDNA is maintained using established mechanisms due to shared characteristics with the
mitochondrial genome. Implications of ecDNA discovery in this organism are wide-reaching due to the potential for new strategies to target resistance development.

352F Molecular composition of the microtub in Toxoplasma Ludek Koreny1, Christen Klingeler2, Brandon Mercado1, Simon Gras3, Simon Butterworth1, Yolanda Rivera-Cuevas4, Vernon Carruthers4, Joel Dacks2, Markus Meissner3, Ross Waller1 1) Department of Biochemistry, University of Cambridge, Cambridge, UK; 2) Division of Infectious Disease, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.; 3) Department of Veterinary Sciences, Experimental Parasitology, Ludwig-Maximilians-Universität, Munich, Germany; 4) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA.

The microtub is a small, cup-shaped depression of the plasma membrane found in many Apicomplexan species and it is believed to be the site of endocytosis. This structure was first described nearly sixty years ago, however its molecular composition is still largely unknown although the presence of clathrin was suggested based on ultrastructural studies. We performed a search of the Toxoplasma proteome for homologues of endocytotic factors known from other eukaryotes and performed proximity biotinylating assays using five of the validated proteins as baits. Altogether we found thirteen proteins that share the same specific location within the Toxoplasma pellicle. Among these are homologues of proteins implicated in endocytosis in other eukaryotic models including all four canonical subunits of the AP-2 adaptor complex, another protein that contains the AP-2 alpha C-terminal domain, an Eps15 homology domain containing protein, a dynamin-related protein, and an ADP-ribosylation factor GTPase-activating protein (Arf-GAP). Our data, however, do not support the involvement of clathrin.

Interestingly, three of the proteins we detected in this structure have been linked to artesiminin resistance in Plasmodium, namely the kelch domain protein 13 (K13), the mu subunit of AP-2 adaptor complex and the deubiquitinase UBP1. Using super-resolution microscopy, we found that the involved proteins form a ring-like structure which is associated with a pore spanning the cell pellicle. The ring structure is sandwiched between two neighbouring alveolar sacks that form the plates of the inner membrane complex and locate here early in the pellicle of the daughter cells. Life-cell imaging together with FRAP experiments suggest little protein recycling and high stability of the proteins involved. This is in contrast with the dynamic nature of endocytic factors that was observed in other eukaryotes. A dedicated and stable site for endocytosis likely represents an evolutionary solution to the problem of transporting molecules from the host cell into the parasite, which is otherwise shielded by the pellicle. The implication of this structure in formation of artesiminin-resistant Plasmodium suggests that modulation of endocytosis is the mechanistic basis of tolerance to this drug. Moreover, endocytosis in apicomplexans is otherwise poorly understood, and the identification of these structures provides a pathway to better understanding the mechanistic basis and importance of this process.

353F ID-ing the Dense Granule Proteome of P. falciparum Manuel Fierro1, Josh Beck1 1) Biomedical Sciences, Iowa State University, Ames, IA.

Apicomplexan parasites are characterized by several unique secretory organelles critical for host cell invasion and subversion including rhoptries, micronemes, and dense granules (DGs). To date, the bulk of the literature regarding these apical structures in Plasmodium has centered around rhoptries and micronemes with only a few proteins localized to the dense granules. These include members of the PTEX complex as well as EXP1, PV1, and RESA with roles including the establishment and maintenance of the parasitophorous vacuole (PV), transport across the encasing PV membrane and remodeling of the host RBC. DGs discharge immediately after invasion and are reformed in daughter merozoites in preparation for egress and the next round of host cell invasion. While DGs are expected to contain key machinery for initial establishment of the PV and the intracellular niche, characterization of the DG proteome is challenging due to the short-lived nature of merozoites and the difficulty in separating DG proteins replenished through the secretory pathway from those initially secreted by the DGs. To overcome these challenges, we genetically modified parasites to permit conditional fusion of TurboID to the C-terminus of the dense granule protein EXP2 using the dimerezire C recombinase, enabling selective targeting of the EXP2-TurboID fusion to merozoite DGs but not the mother PV. This system showcases the power of diCre and TurboID to resolve short-lived events during parasite development and we are currently using this strategy to better define the initial activities involved in establishment of the PV and intracellular niche.

354F Development of Pro-competitive Plasmodium falciparum cytoplasmic prolyl-tRNA synthetase inhibitors Mark A. Tye1,2, N. Connor Payne1,2, Lola Fagbam1,2,3,4, Catrine Johansson5,6, Kritika Singh2, Sofia A. Santos2, Amanda K. Luken4, Udo Oppermann5,6, Dyann F. Wirth3,4, Ralph Mazitschek1,2,3,4,1) Harvard Graduate School of Arts and Sciences; 2) Massachusetts General Hospital Center for Systems Biology; 3) Harvard T.H. Chan School of Public Health; 4) Broad Institute of MIT and Harvard; 5) Structural Genomics Consortium, University of Oxford; 6) Botnar Research Centre, NIHR Oxford Biomedical Research Unit.

The emergence and spread of resistance to first-line antimalarials including chloroquine, atovaquone, pyrimethamine, sulfadoxine, and now artesiminin threatens our ability to treat and contain malaria. This problem is exacerbated by the limited number of targets exploited by current drugs, most of which are only relevant for the asexual blood stage (ABS), limiting their utility to treatment of acute malaria. Therefore, new antimalarial therapies that exploit novel targets and pathways essential for multiple life stages are highly sought for primary prophylaxis and transmission blocking, in addition to acute treatment.

Inhibition of the Plasmodium falciparum cytoplasmic prolyl-tRNA synthetase (PfPRS) has been shown to be a highly promising approach for the development of next generation antimalarials with dual stage activity. However, the rapid evolution of tolerance to halofuginone (HFG) analogues, the most widely studied class of PfPRS inhibitors, through altered host homoeostasis has been concerning for translational development. Further, independent long-term resistance selections with HFG resulted in PfPRS-L482H and PfPRS-L482F mutations that, in combination with the elevated proline levels, conferred high-level HFG-resistance.

To overcome the limitations of HFG analogs, we developed a novel class of pyrazinamide PfPRS inhibitors that are ATP-competitive, proline-competitive (binding affinity increased with increasing proline concentrations), and >25-fold selective for PfPRS over HsPRS. CPM2D6 is our most potent pyrazinamide analog with EC50 = 68 nM for ABS PfDd2. CPM2D6 is active against both HFG-tolerant ABS PfDd2 (EC50 = 116 nM) and HFG-resistant PfPRS-L482H ABS PfDd2 (EC50 = 78 nM). Three independent long-term resistance selections conducted with CPM2D6 did not elicit rapid tolerance as reported for HFG analogs. After continued selection for 47-generations, we observed parasites with 50-fold resistance to CPM2D6 in one of the selections. PCR sequencing of these resistant revealed a mutation in the ATP-binding site of the PfPRS. We are characterizing the effect of this mutation on PfPRS and are designing analogs to that are insensitive to it. We also have solved x-ray crystal structures for CPM2D6 bound to PfPRS and are using them to design additional analogs with improved potency and selectivity. Thus, our pyrazinamide inhibitors represent promising leads for the development of resistance antimalarials.

355F Arginine methylation is tightly regulated throughout Leishmania (Vianna) braziliensis life cycle Angela Cruz1, Lucas Lorenzon1, Juliana Alcforor Diniz1, Rubens Miseraí Magalhães1, Leticia Almeida1, Tiago R. Ferreira2 1) Ribeirão Preto Medical School, University of São Paulo, SP, Brazil; 2) National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, United States.

Control of gene expression in trypanosomatids occurs mainly at the post-transcriptional level, and RNA binding proteins (RBPs) are key players in the determination of transcript fate. Among other post-translational modifiers, RBPs are targets of Protein Arginine Methyltransferases (PRMTs), which transfer a methyl group to arginine residues of proteins affecting their RNA-binding capacities. Herein we present the preliminary characterization of the five predicted arginine methyltransferases of Leishmania braziliensis; overexpression, knockout, and parasites with tagged versions of each PRMT were obtained by CRISPR/Cas9 gene editing and used in the investigation. We observed that monomethylation seems to prevail over dimethylation in Leishmania spp. Profiles of arginine methylated proteins are variable among species, and in L. braziliensis, the arginine methylated protein profiles vary across life cycle stages.
Overexpression and knockdown of some PRMTs led to changes in methylation profiles of *L. braziliensis*. PRMTs are more abundant in proliferative promastigote stages than in metacyclics and amastigotes, and none of them are essential for promastigote growth. PRMT1 knockout combined with all other PRMTs was obtained, and we confirmed that PRMT7, in the absence of PRMT1, scavenges its substrates. Tagged versions of the five *L. braziliensis* PRMTs were used to identify putative targets by co-immunoprecipitation, including several RBPs. Finally, preliminary results indicate that *L. braziliensis* PRMTs may affect the infection profile *in vitro*. Our data indicate that arginine methylation is modulated across life cycle stages in *L. braziliensis*. It suggests possible functional cooperation and overlapping of the different PRMTs towards their targets, suggesting an important regulatory role of these proteins in *L. braziliensis*, which may even affect parasite-host cell interaction.

356F *Trypanosoma brucei* TimS0 Show Connection with Mitochondrial Cardiolipin synthesis and Plays Critical role in Parasite Infectivity Minu Chaudhuri1, Anuj Tripathi1, Ujjal Singh1 1) Department of Microbiology, Immunology, and Physiology. Meharry Medical College,.

TimS0 is a receptor translocase of the TIM23 complex in the mitochondrial inner membrane in fungi and animals. Besides its canonical functions, TimS0 plays roles in stoichiometry, stress tolerance, and developmental regulation in different systems. *Trypanosoma brucei*, a parasitic protozoan and the infective agent for African trypanosomiasis, possesses a homologue of TimS0 (TbTimS0). Unlike its fungal counterpart, TbTimS0 possesses a pair of characteristic DXDX(T/V) phosphate signature motifs. A similar motif exists in proteins of the holoacid holagene family, including phosphatidic acid phosphatase (PAP) that dephosphorylates phosphatidic acid (PA) to diacyl glycerol (DAG). PAP participates in membrane biogenesis and signaling [YS1]. Recent studies showed that some members of this family possess both protein and lipid phosphate activities. Indeed, we found that the recombinant TbTimS0 is able to dephosphorylate both serine/threonine and tyrosin phosphorylated peptides and also to hydrolyze PAP in a concentration-dependent manner, suggesting that TbTimS0 likely possesses both protein and lipid phosphate activities. PA is the primary source for biosynthesis of cardiolipin, a major lipid of the mitochondrial inner membrane. TbTimS0 depletion in the bloodstream form of *T. brucei* reduced cardiolipin levels and decreased mitochondrial membrane potential. TbTimS0 RNAi also significantly reduced cell growth of BF in culture, increased the levels of AMPK phosphorylation, and arrested cell division. Fluoresce microscopy revealed an accumulation of cells with larger kinetoplast and nucleus due to TbTimS0 knockdown. The number of cells with 2N and 2K were reduced significantly and cells with stumpy-like morphology was noted. Importantly, we observed that TbTimS0 depleted parasites were unable to establish infection in rats. Removal of doxycycline from the drinking water of the rats to reverse the RNAi-effect and secondary or tertiary infections with 10-100 folds more parasites also did not increase the blood parasitemia levels more than 10³/ml. Depletion of TbTim17, the major protein translocator of the mitochondrial inner membrane didn’t show a similar phenotype, indicating that TbTimS0 with both protein and lipid phosphate activities plays an important role in mitochondrial membrane biogenesis and parasite infectivity.

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357F High speed, 3D imaging reveals chemotactic behavior specific to human-infective *Leishmania* parasites Rachel Findlay1,2, Mohamed Osman1,3, Kirstin Spence1, Paul Kaye1,3, Laurence Wilcox2, Pégine Walrad1 1) York Biomedical Research Institute, Department of Biology, University of York, UK; 2) Department of Physics, University of York, UK; 3) Hull York Medical School, University of York, UK.

Cellular motility is an ancient eukaryotic trait, ubiquitous across phyla with roles in predator avoidance, resource access and competition. Flagellar-dependent motility is seen in a variety of parasitic protozoans and morphological changes in flagellar structure and function have been qualitatively described during differentiation. However, whether the dynamics of flagellar motion vary across lifecycle stages and whether such changes serve to facilitate human infection is not known. Here we used holographic video microscopy to study the pattern of motility in sandfly midgut procyclic promastigote (PCF) and differentiated human infective metacyclic promastigotes (*META*) forms of *Leishmania* parasites. We discovered that PCF swim in a slow, corkscrew motion around a gently curving axis while META display ‘run and tumble’ behaviour in the absence of stimulus, reminiscent of bacterial behavior. In addition, we demonstrate that META specifically respond to a macrophage-derived stimulus, modifying swimming direction and speed to target host immune cells. Thus, the motility strategy employed by *Leishmania* appears as a random search that is replaced with a ballistic swimming motion in the presence of an immunological stimulus. These findings shed unique insights into how flagellar motion adapts to the particular needs of the parasite at different times in its lifecycle and define a new pre-adaptation for infection of the human host by which the *Leishmania* parasite promotes its own phagocytic uptake.

358F MKT1 interacting RNA-binding protein and its role in the tripanosome Variant Surface Glycoprotein mRNA stabilization Larissa Nascimento1, Franziska Egler1, Katharina Arnold1, Nina Papavasiliou2, Christine Clayton1, Erben Esteban2,3 1) Zentrum für Molekulare Biologie der Universität Heidelberg, Germany; 2) Division of Immune Diversity, Deutsche Krebsforschungszentrum (DKFZ), Heidelberg, Germany; 3) Universidad Nacional de San Martín, Provincia de Buenos Aires, Argentina.

Control of gene expression in kinetoplastids such as trypanosomes depends heavily on RNA-binding proteins (RBPs) that influence mRNA decay and/or translation levels. Several RBPs do this by interacting with MKT1. MKT1 then, on one hand recruits a RNA stabilisation complex (containing PBP1, XAC, L5M12 and an associated poly(A) binding protein, PABP2) while on the other, it interacts with EIF4G5 (a component of the EIf4E-EIf4G5 translation initiation complex). One of the RBPs that interact with MKT1 is CF2B. CF2B is an essential bloodstream-form specific protein that lacks known RNA-binding domains. CF2B is highly enriched in the Variant Surface Glycoprotein mRNA bound proteome and is essential to maintain the VSG mRNA levels. Reporter experiments in procyclic forms show that the action of CF2B depends on a conserved 16-mer element in the VSG 3'-untranslated region, while a conserved 8-mer in the 3'-UTR is implicated in VSG mRNA down-regulation. CF2B interacts with MKT1 via a conserved HDYP motif, mutation of which abolishes CF2B's expression-promoting activity. In addition, CF2B has an N-terminal cyclin-F-box domain. F-box proteins are found in SCF complex (SKP1-Cullin-F-Box), which are E3 ligase components of the ubiquitination machinery. We have demonstrated that the interaction between CF2B and trypanosomape SKP1 depends on the F-box domain and it is involved in auto-regulation of CF2B abundance. Possible Cullin partners and further ubiquitination targets of this SCF-complex are currently under investigation. References: Benz and Clayton, 2007 (PMID: 17920137); Singh et al., 2014 (PMID: 24470144); Nascimento et al., 2020 (PMID: 32532821).

359F Molecular biodiversity and evolution of ascorbate peroxidase in *Trypanosoma cruzi*. Rafael Miranda de Souza1, Lívia Cristina Santos2 1) René Rachou Instituto Oswaldo Cruz Foundation-IRR; 2) Federal Institute of Minas Gerais - IFMG.

Chagas disease, caused by a hemoflagellate parasite called Trypanosoma cruzi, which is transmitted to humans by triatomines, was described in 1909 by Carlos Chagas. Currently, it is estimated that there are eight million people infected around the world. The disease has different clinical manifestations and drug resistance rates, which have been associated with different types of parasite strains. The antioxidant pathway of the *T. cruzi* parasite protects it against oxidative stress generated by the metabolism of the drug or by the host immune system. In this context, our work proposes to conduct a study of the protein ascorbate peroxidase (APX) of *T. cruzi* from an evolutionary perspective. Initially, we conducted the typing of *T. cruzi* strains using molecular markers cytochrome oxidase subunit II, spliced-leader intergenic region and 245s rDNA subunit. Of these, one was classified as Tcl, three as TcII, and two as TcVI. Later, the APX gene of 12 strains of *T. cruzi*, including representatives of the six groups, was amplified by PCR using two pairs of specific primers. The PCR reaction amplified two APX genes for each strain, which were cloned and sequenced. A search was conducted in TriTrypDB using the Pfam identifier (PF00141). This search recovered 92 APX
sequences of trypanosomatids, being one to three sequences found in each taxon. The dataset formed with sequences from TriTrypDB and the ones obtained experimentally were further analysed using the MEGA 6 package. The phylogenetic reconstruction by the maximum likelihood method generated a tree with three clades with high support values (96 and 100%). These clades correspond to the three distinct genes/proteins found in each particular trypanosomatid, named here as, APX1, APX2, and APX3. Each major clade have two clades: one consisting of Trypanosoma sequences and another clade with sequences of Crithidia, Endotrypanum, Leishmania, and Leptomonas. These results suggest that the APX of trypanosomatids is a member of a gene family. Previous studies have described a family of APX in plants, which corroborates our results. Here, we describe for the first time the evolutionary analysis of the APX gene family in trypanosomatids. Furthermore, we did the modelling of some of these proteins using the SWISS-MODEL. The model of these proteins will contribute to studies of their function, helping to understand the antioxidant pathway of T. cruzi. The in silico analysis in DrugBank using sequences of these proteins, showed that the APX1 interact with Isoniazide. We suggest Isoniazide to be tested in association with Benzimidazole against T. cruzi. Our findings will contribute to the design of new studies paving new research approaches for this family of proteins. In a broader sense, our work will contribute to the understanding of this pathway, which is one of the targets in the search for new and more effective drugs for the treatment of Chagas disease.

360F TBRRM1 Has a Duplex DNA Binding Activity That is Required for Telomere Localization and Monoallelic VSG Expression Marjia Afrin1, Amit Gaurav1, Xin Yang2, Xuehua Pan Pan2, Yanxiang Zhao Zhao2, Bibo Li1 1) Cleveland State University, Cleveland, OH; 2) The Hong Kong Polytechnic University, Hung Hom, Hong Kong, PRC.

Trypanosoma brucei causes human African trypanosomiasis and regularly switches its major surface antigen, VSG, to evade the host immune response. VSGs are monoallelically expressed from subtelomeric loci. We have shown that telomere proteins affect VSG silencing and switching. Specifically, TbRAP1 is essential for cell proliferation, associates with the telomere chromatin, is essential for VSG silencing, and suppresses VSG switching. However, how is TbRAP1 localized to the telomere was unknown. Known RAP1 homologs either bind telomere DNA directly through their Myb and Myb-like domains or are recruited to the telomere through their interaction with duplex telomere DNA binding factors. Although TbRAP1 interacts with TbTRF, the duplex telomere DNA binding factor in T. brucei, TbRAP1 is still localized at the telomere in TbTRF depleted cells. TbRAP1 also has a Myb domain, but TbRAP1 is localized to the telomere in a Myb-domain independent manner. We have now identified both a double-strand and a single-strand DNA binding activity in TbRAP1. Both these activities depend on a short, highly positively charged peptide that resides in the Myb-like domain. Two S residues adjacent to this DNA binding patch can be phosphorylated in vivo. Surprisingly, phosphomimicking mutation of the S residues significantly diminishes TbRAP1’s dsDNA binding activity in vitro and abolishes TbRAP1’s telomere localization in vivo. Importantly, TbRAP1’s sDNA binding activity is essential for TbRAP1’s localization at the telomere, VSG silencing, and cell proliferation, indicating that this DNA binding activity is essential for TbRAP1’s telomere functions.

361F Role of TbRRM1 in transcriptional regulation in Trypanosoma brucei Analía Níttolo1, Carolina Baňuelos2, Daniel Sánchez1, Gabriela Levy1 1) Instituto de Investigaciones Biotecnológicas. Buenos Aires, Argentina.; 2) University of Miami. Florida, United State.

Since transcription in trypanosomatids is polycistronic, regulation of gene expression occurs mainly at the post-transcriptional level mediated by RNA binding proteins (RBPs). In our lab we focus on elucidating the function of the RBP TbRRM1 of T. brucei. Previously, we have demonstrated that TbRRM1 is essential for survival in procyclic and bloodstream form stages since its silencing affects the parasite growth curve, produces aberrant phenotypes and promotes cell death by a mechanism compatible with apoptosis. On the other hand, RNA-Seq assays carried out in procyclic cells by Roditi’s lab showed that levels of 1/3 of the transcripts decreased after TbRRM1 depletion and many of them proceed from genes located in a particular region of chromosome 9. These authors also suggested that TbRRM1 could be a chromatin remodeler since the increase of H3 occupancy observed after TbRRM1 depletion. Results from our lab, indicated that TbRRM1 depletion affects RNA Pol II transcription-elongation rate and leads to compacted chromatin in a polycistronic transcription unit located in the same chromosome.

In the present work we showed that TbRRM1 is both recruited to chromatin and to specific RNAs. In addition, we characterized TbRRM1 binding properties in the presence of RNase A, RNase H and Actinomycin D. Because our results suggested that TbRRM1 binds DNA-RNA hybrids, we decided to study the formation of R-loops in TbRRM1 depleted parasites by immunofluorescence with the S9.6 antibody. These experiments showed a significant increase in the number of positive intranuclear dots, thus suggesting that TbRRM1 prevents R-loops accumulation.

Altogether, our results suggest that RNA Pol II transcription-elongation impairment, induced by TbRRM1 depletion, might be a consequence of R-loops accumulation.

362F Understanding and characterization the first steps of proline biosynthesis in Trypanosoma cruzi Leticia Marchese1, Marilene S Braga1, Ariel M Silber1 1) Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP.

Trypanosoma cruzi is the etiologic agent of Chagas Disease. It faces several stress conditions during its life cycle, including starvation. Amino acids are used as energy sources and more in general, to protect the parasite against these stresses. In addition they play important roles in the T. cruzi differentiation from insect replicative forms to the forms able to infect the mammalian hosts. Proline (Pro) has been widely described as a main player in these processes. For example it is involved in ATP production, osmotic control, protection against to nutritional, oxidative and thermal stress, cellular differentiations and cellular invasion. T. cruzi can obtain Pro through its transport, or its biosynthesis from glutamate (Glu). Pro biosynthetic pathway initiates with two reactions catalysed by a bi-functional enzyme, Δ1-pyrroline-5-carboxylate (PSC) synthase (PSCS): 1. the phosphorilation of Glu forming γ-glutamyl phosphate (glutamil kinase domain, GK) and 2. the reduction of γ-glutamyl phosphate to glutamate-γ-semialdehyde (GSA) (glutamil phosphate reductase domain, GPR), oxidizing NADPH. Next, GSA is spontaneously converted into PSC which, as a substrate of a second enzyme, PSC reductase (PSCR), is reduced to Pro. PSCR in T. cruzi has been characterized as a cytosolic NADPH-dependent enzyme. Interestingly, it is regulated by the NADPH concentration via a substrate uncompetitive inhibition. Here we showed a correlation of Pro production in vivo with incubation of different concentrations of Glu. We expressed and purified TcpSCS in its active form in Escherichia coli BL21. Recombinant TcpSCS was used as immunogen to produce specific polyclonal antibodies in mice according to standard protocols. Digitonin permeabilization and immunolocalization experiments showed a cytosolic localization for TcpSCS. We also cloned separately both subunits, corresponding respective GK and GPR domains, which were expressed and affinity-purified. Initial kinetic analysis of the TcGPR subunit was performed by measuring the reverse reaction (using GSA and NADP as substrates) which allowed us to obtain kinetics parameters for the activity (KM(GSA)app = 0.77 ± 0.2 mM and Vmax(GSA)app = 0.73 ± 0.09 µmol NADPH min-1 protein mg-1; KM(NADP+)app = 0.48 ± 0.17 mM, and Vmax(NADP+)app = 0.51 ± 0.06 µmol NADPH min-1 protein mg-1). The better understanding of the enzymological properties of the components of PSCS will allow us to unveil its mechanism.

363F Different roads to search for novel trypanocidal strategies Laura Fraccaroli1, Maria Daniela Ruiz1, Verónica De Pino1, Pablo Torres1, Dario Balcazar1, Luciana Larocca1, Cristina Vanrell2, Patricia Romano2, Carolina Carrillo1 1) ICT Milstein - CONICET, Buenos Aires, Argentina; 2) IHEM - CONICET - UNCuyo, Mendoza, Argentina.

Chagas disease is an endemic parasitosis originally from Latin America, caused by the protozoan Trypanosoma cruzi (T. cruzi). As current therapies (benznidazole and nifurtimox) are limited in efficacy and show multiple side effects, there is a need to identify new effective and specific therapeutic strategies.
In this sense, there are several ways to find potential trypanocidal compounds; the present work focuses on two strategies: 1- the target repurposing and 2- the use of literature and traditional medicine to find new uses to approved drugs or natural compounds.

The strategy 1 compares *T. cruzi* targets with those that are homologues in other organisms (which are characterized and their interaction with drugs is known). In line with this, we study anthracnilcides (DXR-doxorrubicin and DNR-daunorrubicin) for being drugs used in humans and incorporated to cells by the hOCT1 transporter (a low affinity polyamine transporter) and potentially affecting *T. cruzi* polyamine transporter TcPAT12. Our results showed that DXR and DNR affected *T. cruzi* epimastigotes survival and proliferation (EC50 0.1uM for DNR and 2uM for DXR), metacyclogenesis (from 14.8% in control condition to 8.9% with DNR) and replicative capacity of amastigotes (reduced by half in the presence of DNR). These effects could be related both to the decrease of polyamine uptake and intracellular concentration by anthracyclines, and to their cellular toxic effects.

The strategy 2 focussed on the antiparasitic effect of certain drugs or natural compounds reported by literature. One of our ongoing studies is the Melia azedarach (MA) extract, obtained from ripe fruits of the tree, which presents antifungal and antihelminthic properties. The extracts were obtained by different extraction methods using aqueous and organic solvents. Those extracts obtained with DMSO or ethanol showed an antiproliferative effect on *T. cruzi* epimastigotes with EC50 values lower than 1 mg/ml. The extracts maintained their activity when conserved at -20°C while at 4°C it diminished by half. Preliminary HPLC analysis showed that individual fractions of the MA extract did not have an effect in the epimastigotes proliferation, indicating the presence of several bioactive compounds which interact in order to be cytotoxic. Further studies are required to identify and characterize those compounds in the MA extract.

The results shown in this work intend to illustrate some of the approaches used by our research group in order to find and characterize potential trypanocidal drugs. Our analysis strategy starts with the screening of the drugs or extracts in *T. cruzi* epimastigotes cultures and then continues in other stages of the life cycle to assess the infectivity and intracellular replication. Further studies in acute and chronic Chagas disease animal models are required to have a global understanding of how a drug can be postulated as a good candidate to use in the clinic.

364F Disease and mixed species *Theileria* spp. infection among sheep in Oman Salama Al-Hamidi1, Elshafie I. Elshafie2,3, Ghalip Mohammed 4, Naseeb Al-Saqiri2, Hamza Babiker1 1) Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, Oman; 2) Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Oman; 3) Central Veterinary Research Laboratory, Al Amanat, P.O. Box 8076, Khartoum, Sudan; 4) Alfalah Veterinary Clinic, Oman.

Mixed species infections of *Theileria* spp. are common in nature, epidemiological surveys in Oman revealed high prevalence of mixed infection comprising, the pathogenic (*T. lestoquardi*) and non-pathogenic (*T. ovis*) among apparently healthy sheep (Al-Fahdi et al., 2017). Experimental and epidemiological data suggest that mixed species infections elicit cross-immunity that can modulate pathogenicity and disease burden at the population level (Woolhouse et al., 2015). Our most recent data illustrate a potential competitive interaction between the two ovine *Theileria* spp, and a substantial reduction in the risk of mortality in mixed parasite infections, indicating that *T. ovis* confers heterologous protection against lethal *T. lestoquardi* infection (Awad et al., 2020). The present study extends the above findings and examine disease correlates (clinical markers, hematology parameters and density of parasites) among mixed and single infection. PCR and qPCR of the 18S rRNA gene were used to detect and quantify *Theileria*. Overall *Theileria* spp infection was 75(19.2) out of 390 sheep, 16.6%, 2.1% and 0.5%, harbored, mixed-infections of *T. lestoquardi* and *T. ovis*, *T. lestoquardi* alone or *T. ovis* alone, respectively. The overall densities of both parasite species were higher as single infection vs mixed infection, and there was a higher relative density of pathogenic *T. lestoquardi* in mixed infection, indicated a competitive advantage over the non-pathogenic *T. ovis*. However, there was no difference in density or hemotological indices between of *T. lestoquardi* (single infection) and mixed infection. Though, mix infection (*T. ovis* and *T. lestoquardi*) had significantly lower eosinophils. In addition, there was significant positive effect of *T. lestoquardi* (mix infection) density on platelet.

The above results corroborate our previous findings of competitive interaction between the two ovine *Theileria* spp. The substantial prevalence of mixed infection compared to single infection support the hypothesis of reduction in the risk of mortality in mixed parasite infections, indicating that *T. ovis* confers heterologous protection against lethal *T. lestoquardi* infection. However, there was no differences in disease markers among sheep with single and mixed species infection.

365F Misregulation of Alba domain-containing proteins in *Plasmodium falciparum* blood stages differentially affects growth and the parasite transcriptome Dimple Acharya1, Vishnu V. Ashok1, Cameron Macpherson2,3,4, Shubhada Hegde1, Artur Scherf2,3,4, Shruthi Vembar1 1) Robert Koch-Institute, Berlin, Germany; 2) Helmholtz Centre for Infection Research and Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany.

The malaria parasite *Plasmodium falciparum* adopts over ten different morphological and physiological states as it develops within its human host and mosquito vector. To achieve each state, this unicellular pathogen transcriptionally and post-transcriptionally regulates the expression of its ~5700 genes, many of which are unique to the *Plasmodium* genus and act as virulence factors to establish malaria pathogenesis. With atypical RNA-binding domains accounting for a large portion of mRNA-binding proteins (mRBPs) in *Plasmodium spp.*, our broad interest is to elucidate their role in blood stage gene regulation; in this study, we focus on the DNA/RNA-binding Alba domain, which is thought to be of archeal origin. We previously described four Alba domain-containing proteins, PfAlba1, PfAlba2, PfAlba3 and PfAlba4 in *Plasmodium* spp. are common in nature, epidemiological surveys in Oman revealed high prevalence of mixed infection comprising, the pathogenic (*T. lestoquardi*) and non-pathogenic (*T. ovis*) among apparently healthy sheep (Al-Fahdi et al., 2017). Experimental and epidemiological data suggest that mixed species infections elicit cross-immunity that can modulate pathogenicity and disease burden at the population level (Woolhouse et al., 2015). Our most recent data illustrate a potential competitive interaction between the two ovine *Theileria* spp, and a substantial reduction in the risk of mortality in mixed parasite infections, indicating that *T. ovis* confers heterologous protection against lethal *T. lestoquardi* infection. However, there was no differences in disease markers among sheep with single and mixed species infection.

**366F** The mode of action of *T. gondii* tissue cyst inhibitors Deborah Maus1, Elyzana Putrianti1, Jennifer Herrmann2, Rolf Müller2, Martin Blume1 1) Robert Koch-Institute, Berlin, Germany; 2) Helmholtz Centre for Infection Research and Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany.

The intracellular, apicomplexan parasite *Toxoplasmosis gondii* infects up to 30% of the global human population and causes life-threatening diseases in immuno-compromised patients. Chronically persisting bradyzoites form cysts in brain and muscle tissues and are responsible for transmission and remission of this disease. However, currently available medical treatment options including many novel developmental treatments are only effective against the virulent tachyzoites but fail to clear the chronic stages of *T. gondii*.
To address this shortcoming, we screened a set of myxobacterial compounds and the MMV PathogenBox against tachyzoites and matured, pan-resistant in vitro tissue cysts. We have identified 51 inhibitors that are effective against tachyzoites and 33 against bradyzoites. Interestingly, only 19 compounds inhibit growth of both forms of the parasite underlying their divergent biology.

To investigate the mode of action of these substances we developed an untargeted metabolomics approach using HILIC-UHPLC-MS. We detect a complex metabolome encompassing both known and unsuspected metabolites in *T. gondii*. Based on these data we compare the response of the parasite to candidate drugs to established inhibitors with known modes of action. So far we were able to determine the metabolic phenotype of derivatives of Aurachin and Chlorotolin. Aurachin shares 30 commonly regulated metabolites with Atovaquone and HDQ, indicating inhibition of mitochondrial bc1-complex in the electron transport chain, while the metabolic signature of Chlorotolin suggests an unrelated mode of action. Our findings support an essential function of the mitochondrial bc1-complex in *T. gondii* bradyzoites.

In conclusion, our work identifies promising compounds that are suited for the development of future chemotherapy agents as well as for the functional interrogation of the metabolism of *T. gondii* tissue cysts.

367F Identification of a nuclear protease that is associated to histone H3 clipping and DNA replication in blood stages of malaria parasites *Plasmodium falciparum*.

In summary, our results suggest a possible role of TfMOB1 protein in a correct parasite division.

Interestingly, a significant increase of multinucleated cells (3-4 nuclei), 22% and 35%, were found in TfMOB1full length-HA and TfMOB1ΔNt-HA transfected parasites, respectively, compared to 7.2% in EpNeo parasites (control); and for multinucleated cells with ≥ 4 nuclei, 6.3%, 16% and 1.3% were found in TfMOB1Fl-HA and TfMOB1ΔNt-HA and EpNeo transfected parasites, respectively. Additionally, we observed nuclear abnormalities such as micronuclei, micronuclei cluster, nucleolar bridges and apoptosis. Based on these data we compare the response of the parasite to


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368F Resolvin D1 Administration is Beneficial in *Trypanosoma cruzi* Infection

Chagas disease is a major public health issue, affecting ~10 million people worldwide. Transmitted by a protozoan named *Trypanosoma cruzi* (*T. cruzi*), this infection triggers a chronic inflammatory process that can lead to cardiomyopathy (Chagas Disease). Resolvin D1 (RvD1), is a novel pro-resolution lipid mediator whose effects on inflammatory diseases dampens pathological inflammatory responses and can restore tissue homeostasis. Current therapies are not effective in altering the outcome of *T. cruzi* infection, and as RvD1 has been evaluated to be a therapeutic agent in various inflammatory diseases, we examined if exogenous RvD1 could modulate the pathogenesis of Chagas Disease in a murine model. CD-1 mice infected with *T. cruzi* Brazil strain were treated with RvD1. Mice were administered 3µg/kg RvD1 intraperitoneally on days 5, 10, and 15 to examine the effect of RvD1 on acute disease or administered the same dose on days 60, 65, and 70 to examine its effects on chronic infection. RvD1 therapy increased the survival rate and controlled parasite replication in acute infection and reduced the levels of IFN-γ and TGF-B in chronic infection. In addition, there was an increase in IL-10 with RvD1 therapy in both acute and chronic infection, and a decrease in TGF-B and collagen content in the cardiac tissue. Together, these data indicate that RvD1 therapy can dampen the inflammatory response, promote resolution of *T. cruzi* infection, and prevent cardiac fibrosis.

369F Analysis of the Hippo pathway role in Trichromonas foetus cell division

Bovine trichromoniasis is a venereal disease caused by the extracellular protozoan Trichromonas foetus, which results in significant economic loss in livestock from different regions of the world. While it is known that cell division is a key process for the survival of cells, there are still no reports on the molecular regulation of this process in trichomonads. "Hippo" pathways are ancient protein kinase signaling systems that control cell proliferation and morphogenesis; in these pathways, the protein kinases of the NDR/LATS family are associated with co-activators proteins or "MOB proteins". MOB1 is a central protein for the exit of mitosis being essential for the correct transition from metaphase to anaphase and proper balance between cell proliferation and cell death. Previously, has been reported that when regulate their interactions with the MST1/2 and LATS kinases. Based on this, we investigated the relevance of the phosphorylatable residues present in the extreme N-terminus of the TFM01 protein (OHS93373.1). To this end we transfected TFM01Flfull-length-HA and TFM01ΔNt-HA (lacking a phosphorylation sites 9 and 31) in parasites. By immunofluorescence assay TFM01Flfull-length-HA and TFM01ΔNt-HA showed a localization pattern of nuclear and cytoplasmic. Also, our results demonstrated that MOB1full-length-HA and MOB1ΔNt-HA transfected parasites showed abnormal growth. Interestingly, a significant increase of multinucleated cells (3-4 nuclei), 22% and 35%, were found in TFM01Flfull-length-HA and TFM01ΔNt-HA transfected parasites, respectively, compared to 7.2% in EpNeo parasites (control); and for multinucleated cells with ≥ 4 nuclei, 6.3%, 16% and 1.3% were found in TFM01Fl-HA and TFM01ΔNt-HA and EpNeo transfected parasites, respectively. Additionally, we observed nuclear abnormalities such as micronuclei, micronuclei cluster, nucleolar bridges and apoptosis. In summary, our results suggest a possible role of TFM01 protein in a correct parasite division.

370F Investigation of protein acetylation function in *Leishmania*: Zn-dependent lysine deacetylases as model of study

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Protein acetylation plays a relevant role in regulating essential cellular processes in different organisms. Recently, our group characterized the acetyloyne, set of acetylated proteins, of *Trypanosoma cruzi* and *Trypanosoma brucei*. We observed significant differences in the protein acetylation of enzymes related to the oxidative stress and glycolytic pathway during their life cycles. These characteristics corroborate with the adaptation profile of these parasites during their life cycles, and suggest that this modification may also play a relevant role in other family members, such as *Leishmania sp*. Acetylation levels of proteins are regulated by the activity of two enzyme families: lysines acetyltransferases (KATs), which promote the addition of the acetyl group to lysine residues, and lysines deacetylases (KDAC), which catalyzes the removal of these groups. KDACs can be divided into two main classes: zinc-dependent (classical) and NAD+-dependent (sirtuins). Trypanosomatids have members of both families described in their genome, although in a reduced number compared to mammals, with four zinc-dependent (DAC1, DAC3, DAC4 and DAC5), and three NAD+-dependent (Sir2rp1-3) members. To expand our knowledge on the function of protein acetylation in Trypanosomatids, in this work we began to characterize the role of DACs of *Leishmania mexicana*. Using CRISPR/Cas9 technology, we generated knockouts and endogenous tagged parasite strains for the four DACs genes of the parasite and found that DAC1 and DAC3 are essential in promastigote forms, while DAC4 and 5 are not. Also, using the tagged parasites we observed that DAC1 and 5 are cytoplasmatic, while DAC3 and 4 have nuclear localization. Initial phenotype screening had shown that DAC3 and DAC5 affect promastigote growth and differentiation to infective forms. New experiments are underway to better understand the role of these enzymes in the biology of *Leishmania mexicana*.

**371F Molecular epidemiology of resistance markers of Artemisinin-based Combination Therapies among *Plasmodium falciparum* isolates in Lagos, Nigeria. Olusola Aijayi1, Chinaza Nnam1, Ikenechukwu Nwanwo1 1) Nigeria Institute of Medical Research.**

A major threat to malaria elimination is the increasing spread of drug resistance especially resistance to Artemisinin-based Combination Therapies (ACTs)-the globally approved protocol used for uncomplicated malaria. Very recently, the emergence and clonal expansion of artemisinin-resistant *Plasmodium falciparum* (*P. falciparum*) kelch13 R561H mutant parasites have been reported in endemic regions in Africa. Also, we have previously reported in vivo evidence of Artemisinin-tolerant *P. falciparum* parasites and non-kelch13-related delayed parasite clearance among populations in Lagos, Nigeria. Most reports on molecular surveillance of antimalarial drug resistance have been exclusively on either parasite or host-mediated resistance. However, phenotypic appearance of drug resistance involves the interplay of host and the parasite factors. We hypothesize that coordinated frequency spectrum of *P. falciparum* molecular markers of resistance, chloroquine resistance transporter (*PfCRT*) and *P. falciparum* multi-drug resistance-1 gene (*Pfmdr-1*), and human cytochrome P450 enzymes polymorphisms are important in the appearance of artemisinin resistance. Currently, Nigeria contributes 25% of the global burden of malaria, with unique parasite genetic structures. Therefore, this study profiles the status and frequency spectrum of both *P. falciparum* and host-mediated resistance markers in Lagos, Nigeria.

Genomic DNA from fifty microscopically-confirmed *P. falciparum* samples in a prospective cross-sectional study in Lagos, Nigeria were analysed to genotype the frequencies of human *CYP2B6* and *CYP3A4* alleles, as well as *PfCRT* K76T and *Pfmdr-1* N86Y. Molecular profiling was done by restriction fragment length polymorphism and amplicon sequencing for *CYP3A4*.

The allele frequencies analysis of mutant *PfCRT* K76T and *Pfmdr-1* N86Y alleles revealed 94% and 41% respectively while *CYP2B6* and *CYP3A4* drug resistance variants had frequencies of 20% and 60% respectively in the population studied. Genetic analysis on the sequenced *CYP3A4* revealed that the allele is not under selection pressure, with a Tajima’s D value of -1.23. The high frequencies of antimalarial drug resistance-associated alleles observed in the study underscore the significance and urgency of surveillance of ACT resistance in Nigeria. This will inform policy on national antimalarial use as an important component of malaria elimination strategy.

**372F Alternative approaches for control of gene expression in trypanosomatids. Gabriela Niemirowicz1, Vanina Alvarez1, León Bouvier1 1) Instituto de Investigaciones Biotecnológicas.**

Regulatable gene expression in trypanosomatids relies on pre-establishing cell lines modified to express exogenous regulatory elements such as bacterial repressors and polymerases and therefore, inducible experimentation is frequently restricted to few laboratory strains. Moreover, extending these methodologies to other strains usually requires several additional transfection steps as well as lengthy periods in culture thus affecting the natural wild type properties sought after in field isolates.

Here we explored alternative strategies for inducible transgene expression in *Trypanosoma cruzi* and *Trypanosoma brucei*. We studied the drug dependent modulation of GFP mRNA stability by insertion of a cis-acting riboswitch. This element consists of a *Schistosoma mansoni* hammerhead ribosome fused to a tetracycline aptamer. Reporter GFP expression could be downregulated by tetracycline addition, in *T. cruzi* epimastigotes as well as in procyclic *T. brucei* trypanosomes. Thus, our results would suggest that these riboswitches could be used as alternative tools to study essential genes in trypanosomatids.

**373F Towards development of an auxin-inducible degron system for use in *Plasmodium falciparum* Madeline Farringer1, John Beck1, Joshua Beck1 1) Iowa State University.**

Malaria is a devastating disease that causes nearly half a million deaths annually. While deeper understanding of unique parasite biology is urgently needed to provide novel therapeutic approaches, functional genetic studies are challenging given the limited tools available. We are adapting the plant auxin-inducible degron (AID) to study the function of essential genes in *Plasmodium falciparum*. In its endogenous context, the plant hormone auxin binds an AID sequence present in certain proteins, enabling recognition by a cognate F-box adaptor protein within the Skp1-Cullin-F-box protein (SCF) complex. SCF then recruits an E2 ubiquitin ligase, leading to ubiquitination and rapid proteasomal degradation of the target protein. Due to the high conservation of SCF among eukaryotes, repurposing this system for knockdown in an exogenous context requires only the expression of an auxin-sensitive F-box protein and fusion of the AID sequence to a target protein. Indeed, the AID system has been successfully adapted in Toxoplasma and Plasmodium berghei to study endogenous protein function. While the AID system has also been shown to activate degradation of a fluorescent reporter protein in *P. falciparum*, it has not been applied to endogenous proteins. We have developed AID systems in *P. falciparum* based on TIR1 and AFB2, two different auxin-sensitive F-box proteins, and are evaluating their capabilities with a panel of essential protein targets with diverse biochemical properties, including soluble, integral membrane and secreted proteins. To further optimize these systems, additional F-box adaptor-AID sequence pairs shown to reduce background degradation in mammalian systems are also being tested. Successful establishment of the AID system will accelerate functional genetic studies in *P. falciparum*. Ongoing results will be presented.

**374F Molecular prevalence of intestinal parasites infections in children with diarrhea in Franceville, Southeast of Gabon Sandrine L OYEGUE1, 2, Nal K Ndjangangoye1, 2, Lady C Kouna2, Gwladys M Lekofo2, Franck Mounioko3, Sylvie K Nolna4, Jean-Bernard Lekana-Doukou2, S J 1) Ecole Doctorale Régionale d’Afrique Centrale en Infectiologie Tropicale, Université des Sciences et Techniques de Masuku; 2) Centre International de Recherches Médicales de Franceville (CIRMF); 3) Laboratoire d’Ecologie Vectoriel, Institut de Recherche en Ecologie Tropicale; 4) Capacity for Leadership Excellence and Research; 5) Département de Parasitologie-Mycologie, Médecine Tropicale, Faculté de Médecine, Université des Sciences de la Santé.**

**Background:** Pediatric diarrhea caused by a range of pathogens, including intestinal parasites, is one of main causes of death among children under 5 years of age. The distribution of these parasitic infections overlaps in many environmental, socioeconomic and epidemiological settings. Their distribution and
prevalence varies from region to region. In the current study, we assess the prevalence of intestinal parasites among pediatric patients with syndromic diarrheal disease living in Franceville, Gabon.

Methods: A cross-sectional study conducted in the Amissa Bongo Regional Hospital and Chinese-Gabonese Friendship Hospital in Franceville, between November 2016 and August 2017, enrolled a total of 100 diarrheic children between 0 and 180 months of age. Parasite detection in stool samples was performed using molecular diagnostic by PCR. Difference in means were tested by Student’s t test and ANOVA while principal component analysis was used to determine the correlation between parasite distributions and age groups.

Results: The overall prevalence of intestinal parasite infection was 61% (61/100). Hymenolepis sp and Cryptosporidium hominis/parvum were the most common parasites (31 and 19%, respectively), followed by Encephalitozoon intestinalis (15%), Trichuris trichiura (4%), Dientamoeba fragilis (4%), and Enterocytozoon bieneusi (2%). The polyparasitism rate was 19.7%, with 83.3% double and 16.7% triple infections. Protozoan infections (66.7%) were more prevalent than helminths infections (33.3%). Seasonal association of the circulation of intestinal parasite was statistically significant ($p=0.03$). Correlations between different parasites was also observed.

Conclusion: The prevalence of intestinal parasitic infections is highest in diarrheic pediatric children. The prevalence of parasitic infections indicates that protozoa and helminths are the most common parasites in the Franceville environment. This study reinforces the importance of routine examination of diarrheic stool samples for the diagnostic of intestinal parasites. Further analyses are required to better understand the local epidemiology and risk factors associated with the transmission of intestinal parasites in Franceville, Gabon.

Keywords: diarrhea, children, intestinal parasitic infections, molecular diagnostic, Franceville, Gabon