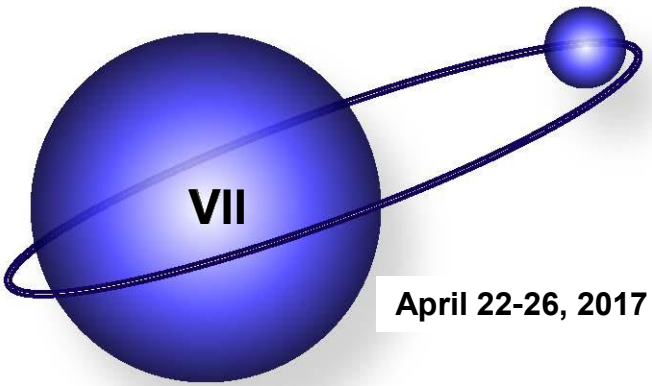
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory · Woods Hole · MA · USA



April 22-26, 2017

KMCBM 2017 Acknowledgements

Seventh Kinetoplastid Molecular Cell Biology Meeting, April 22 – 26, 2017

Organized by Christian Tschudi (Yale School of Public Health, New Haven, USA)

Hosted by the Marine Biological Laboratory, Woods Hole, Massachusetts, USA

The organizer wishes to thank:

The Program Committee:

Barbara Burleigh (Harvard T. H. Chan School of Public Health, Boston, USA)
Nicola Carter (Pacific University, Hillsboro, USA)
Vivian Bellofatto (Rutgers New Jersey Medical School, Newark, USA)
Mark Field (University of Dundee, Dundee, Scotland, UK)

The Staff at MBL:

Katy Dern and her staff for registration and housing; Chris Dematos for website design, web registration and abstract book preparation; All the IT AV Support staff and the staff in Sodexho Food Service at the MBL.

Cover Design: Markus Engstler

KMCBM 2017 Program

Saturday, April 22

02:00 – 05:00	Arrival, Registration and Poster Session A setup
04:00 – 06:30	Greeting and Dinner
07:00 – 09:00	Session I: Cell Biology I (chair: Scott Landfear)
09:00 – 11:00	Mixer

Sunday, April 23

07:00 – 08:30	Breakfast
08:45 – 11:45	Session II: Host-Parasite Interactions (chair: Sam Alsford)
12:00 – 01:30	Lunch
02:00 – 04:30	Session III: Gene Expression/Translational Control (chair: Pegine Walrad)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session A
09:00 – 11:00	Mixer & Poster A/B Changeover

Monday, April 24

07:00 – 08:30	Breakfast
08:45 – 11:45	Session IV: Biochemistry/ Drug discoveries (chair: Dan Zilberstein)
12:00 – 01:30	Lunch
01:30 – 06:00	Free Time
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session B
09:00 – 11:00	Mixer & Poster B/C Changeover

Tuesday, April 25

07:00 – 08:30	Breakfast
08:45 – 11:45	Session V: RNA Biology (chair: Sara Zimmer)
12:00 – 01:30	Lunch
02:00 – 04:45	Session VI: Cell Biology II (chair: Ziyin Li)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session C
09:00 – 11:00	Mixer

Wednesday, April 26

07:00 – 08:30	Breakfast
08:45 – 11:15	Session VII: Molecular Biology (chair: Vivian Bellofatto)
12:00 – 01:30	Lunch or lunch boxes

ROOMS MUST BE VACATED BY 10:00 AM ON Wednesday

SESSION I: Cell Biology I
Scott Landfear, Chair.
Saturday 7:00 pm

1A From solitary swimmers to swarms and back - trypanosomes on their journey through the tsetse fly
***Schuster, Sarah**; Krüger, Tim; Subota, Ines; Thusek, Sina; Rotureau, Brice; Kollmannsberger, Philip; Beilhack, Andreas; Engstler, Markus*

1B Swim like your lifecycle depends on it: The impact of Leishmania flagellar motility
***Findlay, Rachel**; Gadelha, Hermes; Rogers, Matthew; Wilson, Laurence; Walrad, Pegine B*

1C Discovery of new Leishmania motility mutants in a high-throughput CRISPR-Cas9 knockout screen of the flagellar proteome
***Beneke, Tom**; Madden, Ross; Demay, Francois; Valli, Jessica; Wheeler, Richard; Sunter, Jack; Gluenz, Eva*

1D Molecular and systems analysis of cell-cell communication and social behavior in Trypanosoma brucei
***DeMarco, Stephanie**; Stream, Alexandra; Jahanbakhsh, Shahriyar; Hill, Kent*

1E Inositol phosphates regulation of Trypanosoma brucei life cycle developmental progression
***Cestari, Igor;** Anupama, Atashi; Stuart, Ken*

1F A protein kinase A (PKA) signaling pathway mediates promastigote-to-amastigote differentiation in Leishmania
***Zilberstein, Dan**; Fischer Weinberger, Renana; Koren Nitzan, Roni; Bar Avraham, Yael; Myler, Peter, J.*

1G Conservation of quorum-sensing signal responses and cross-species interactions between Trypanosoma brucei and Trypanosoma congolense
***Silvester, Eleanor**; Matthews, Keith*

1H Advances in the identification of the stumpy induction factor (SIF)
***Subota, Ines**; Engstler, Markus; Fekete, Agnes; Muller, Martin J.*

SESSION II: Host-Parasite Interactions
Sam Alsford, Chair.
Sunday 8:45 am

2A Dissecting Leishmania metabolism and population heterogenity in infected tissues
McConville, Malcolm*; Saunders, Eleanor; Kloehn, Joachim; Sernee, Fleur; Ralton, Julie;*

2B TcMcS-Parasite Pressure Gauge: Regulatory role of a mechanosensitive channel in T. cruzi physiological mechanisms
Dave, Noopur; *Barerra, Patricia; Cetiner, Ugur; Sukharev, Sergei; Jimenez, Veronica*

2C Tracking single Trypanosoma cruzi parasites to reservoir sites in the gut during chronic murine infections
Taylor, Martin C.; *Fortes-Francisco, Amanda; Ward, Alexander; Jayawardhana, Shiromani; Lewis, Michael; Kelly, John*

2D Study of T. cruzi intracellular organization reveals a new host-parasite interaction mediated by the amastigote flagellum.
Lentini, Gaelle*; Burleigh, Barbara*

Coffee Break

2E Adipose tissue is a reservoir of Trypanosoma brucei parasites throughout infection
Trindade, Sandra

2F APOLIPOPROTIEN L-1 in Trypanosome Lytic Factors is highly polymorphic in primates, which governs broad resistance
Verdi, Joey; *Thomson, Russell; Raper, Jayne*

2G Role of host triglycerides in Trypanosoma cruzi amastigote lipid metabolism
Martin, Jessica L.; *Gazos-Lopes, Felipe; Burleigh, Barbara*

TT1 FRET Flow Cytometry and Microscopy for Monitoring Glucose in Trypanosoma brucei
Christensen, Kenneth A.; *Voyton, Charles; Ackroyd, Christine; Morris, Meredith; Morris, James (Poster #1)*

SESSION III: Gene Expression/ Translation Control
Pegine Walrad, Chair.
Sunday 2:00 pm

3A Variant Surface Glycoprotein gene expression and monoallelic exclusion: how is this controlled?
Rudenko, Gloria

3B Genome-wide chromosome conformation capture (Hi-C) reveals strong compartmentalization of the Trypanosoma brucei genome
Müller, Laura SM*; Cosentino, Raúl O; Förstner, Konrad U; Lajoie, Bryan R; Kaplan, Noam; Belton, Jon-Matthew; Janzen, Christian J; Steinbiss, Sascha; Otto, Thomas D; Berriman, Matthew; Sebra, Robert P; Dekker, Job; Siegel, T Nicolai*

3C Circadian rhythm controls metabolism in Trypanosoma brucei
Rijo-Ferreira, Filipa*; Pinto-Neves, Daniel; Barbosa-Morais, Nuno; Takahashi, Joseph; Figueiredo, Luisa*

3D Patterns of chromosome copy number change during growth of Leishmania reveal a link between DNA replication and mosaic aneuploidy
Campbell, Samantha; *Duncan, Samuel; Krasilnikova, Marija; Mottram, Jeremy; McCulloch, Richard; Dickens, Nicholas*

TT2 Variant antigen profiling: a tool for genome-wide analysis of Variant Surface Glycoprotein diversity in Trypanosoma congolense
Silva Pereira, Sara*; Casas, Aitor; Haines, Lee; Noyes, Harry; Sanders, Mandy; Berriman, Matthew; Acosta-Serrano, Alvaro; Jackson, Andrew (Poster #2)*

TT3 Trypanosoma brucei RAP1 suppresses VSG switching by repression of telomere transcription and telomere RNA:DNA hybrids
Nanavaty, Vishal P.*; Sandhu, Ranjodh; Jehi, Sanaa; Pandya, Unnati; Li, Bibo (Poster #3)*

TT4 Puzzling together the Trypanosoma brucei Lister 427 genome
Cosentino, Raúl O.; *Müller, Laura SM; Förstner, Konrad U.; Lajoie, Bryan R.; Kaplan, Noam; Belton, Jon-Matthew; Steinbiss, Sascha; Otto, Thomas D.; Berriman, Matthew; Sebra, Robert P.; Dekker, Job; Siegel, T. Nicolai (Poster #4)*

Coffee Break

3E Elucidating the molecular mechanism for cell cycle-dependent protein translation initiation in trypanosomes
AN, TAI*; LI, ZIYIN*

3F The trypanosome mRNA decapping enzyme is an ApaH-like phosphatase
Kramer, Susanne; *Goos, Carina*

3G Leish4E-IP2 - a novel LeishIF4E-interacting protein involved in stage differentiation
Tupperwar, Nitin; *Meleppattu, Shimi; Gilad, Ayelet; Leger-Abraham, Melissa; Wagner, Gerhard; Shapira, Michal*

3H A novel 'VSG stress response' pathway leads to upregulation of a subset of ER and Golgi proteins during a global translation arrest induced by a VSG synthesis block

Ooi, Cher-Pheng*; Wand, Nadina; D'Archivio, Simon; Gadelha, Catarina; Wickstead, Bill; Rudenko, Gloria*

SESSION IV: Biochemistry/ Drug Discovery
Dan Zilberstein, Chair.
Monday 8:45 am

4A A mitochondrial fucosyltransferase is essential for the viability of Leishmania major
***Beverley, Stephen M.**; Guo, Hongjie; Damerow, Sebastian; Penha, Luciana; Zegzouti, Hicham; Ferguson, Michael A.J.*

4B Assembly and function of BILBO1, a multidomain cytoskeletal protein at the flagellar pocket collar of Trypanosoma brucei
Dong, Gang

4C Aquaglyceroporin-null trypanosomes display glycerol transport defects and respiratory-inhibitor hypersensitivity
***Jeacock, Laura**; Baker, Nicola; Wiedemar, Natalie; Mäser, Pascal; Horn, David*

4D A chemical tool to study signaling from the unconventional PKA-like kinase of Trypanosoma brucei
***Bachmaier, Sabine**; Kramer, Susanne; Githure, George; Volpato, Yuri; Pepperl, Julia; Dupuy, Jean-William; Bonneu, Marc; Genieser, Hans-Gottfried; Schwede, Frank; Boshart, Michael**

Coffee Break

4E A potential mode of action of the anti-trypanosomal benzoxaborole AN7973
***Begolo, Daniela**; Barrett, Michael P.; Clayton, Christine*

4F FRET Flow Cytometry and Microscopy for Monitoring Glucose in Trypanosoma brucei
***Christensen, Kenneth A.**; Voyton, Charles; Ackroyd, Christine; Morris, Meredith; Morris, James*

4G Selective inhibition of RNA polymerase I transcription as a potential approach to treat African trypanosomiasis
***Kerry, Louise Elizabeth**; Pegg, Elaine E; Cameron, Donald P; Budzak, James; Poortinga, Gretchen; Hannan, Kate; Hannan, Ross D; Rudenko, Gloria*

4H Glycosomal hypertrophy and the response to suramin in African trypanosomes
***Zoltner, Martin;** Vaughan, Sue; Gadelha, Catarina; Leung, Ka-Fai ; Güther, Maria Lucia S.; Burrell, Alana; Ali, Liaqat; Ferguson, Michael A. J.; Field, Mark C.*

SESSION V: RNA Biology
Sara Zimmer, Chair.
Tuesday 8:45 am

5A A role for retrotransposon hotspot proteins in transcription in Trypanosoma brucei?
Florini, Francesca; *Naguleswaran, Arunasalam; Bringaud, Frédéric; Roditi, Isabel*

5B The vault RNA of Trypanosoma brucei is required for the production of trans-spliced mRNA
Kolev, Nikolay G.; *Rajan, K. Shanmugha; Tycowski, Kazimierz T.; Toh, Justin Y.; Shi, Huafang; Lei, Yuling; Michaeli, Shulamit; Tschudi, Christian*

5C Dual pseudouridylation on rRNA and snRNAs and the role of hyper-pseudouridylation during the life cycle of Trypanosoma brucei
Michaeli, Shulamit; *Rajan, K. Shanmugha; Chikne, Vaibhav; Doniger, Tirza; Cohen Chalamish,, Smadar; Unger, Ron; Tschudi, Christian*

5D Metabolism and the maxicircle genome: exploring potential relationships using Trypanosoma cruzi strain variability
Kalem, Murat C.; *Flegontov, Pavel; Gerasimov, Evgeny; Rasmussen, Bailey; Susa, Emily; Zimmer, Sara L.*

Coffee Break

5E Early life of mitochondrial RNAs in Trypanosoma brucei
Sement, Francois*; Zhang, Liye; Afasizheva, Inna; Afasizhev, Ruslan*

5F A catalytic RNA helicase subcomplex in the trypanosome holo-editosome
Doharey, Pawan*; Kumar, Vikas; Gulati, Shelly; Wohlschlegel, James; Schnaufer, Achim; Mooers, Blaine; Cruz-Reyes, Jorge*

5G The Leishmania mRNA-bound proteome changes throughout the lifecycle; candidate regulators of parasite differentiation
Walrad, Pegine B.; *de Pablos Torro, Luis M.; Ferreira, Tiago R.; Dowle, Adam;*

TT5 Mutagenesis screens identify editosome protein domains that differentially affect RNA editing between life cycle stages of Trypanosoma brucei
McDermott, Suzanne M.; *Carnes, Jason; Stuart, Kenneth (Poster #88)*

TT6 RNA-binding protein capture reveals a diversity of poly(A)+ RNA-binding proteins in insect-form Trypanosoma brucei
Fisher, Michael; *Das, Anish; Bellofatto, Vivian (Poster #89)*

TT7 High-efficiency chemical-mediated transfection of Trypanosoma cruzi
Olmo, Francisco*; Rotger, Carmen; Taylor, Martin C; Costa, Fernanda C; Costa, Antonio; Kelly, John M (Poster #90)*

SESSION VI: Cell Biology II
Ziyin Li, Chair.
Tuesday 2:00 pm

6A Differentiation and quorum sensing in bloodstream form African trypanosomes
Keith R. Matthews

6B Life-stage specific cargo receptors facilitate GPI-surface coat protein transport in Trypanosoma brucei
Kruzel, Emilia K.; *Zimmett, George; Bangs, James*

6C A GPR89 family surface protein is an oligopeptide transporter that signals slender to stumpy differentiation in T.brucei
Rojas, Federico; *Thompson, Joanne; R. Matthews, Keith*

6D Tubulin poly-glutamylation modulates intraflagellar transport in the Trypanosoma brucei flagellum
Lemos, Moara; *Jung, Jamin; Blisnick, Thierry; Fort, Cécile; Carsten, Janke; Bastin, Philippe*

Coffee Break

6E A molecular model of the mitochondrial genome segregation machinery in Trypanosoma brucei
Hoffmann, Anneliese; *Käser, Sandro; Schneider, André; Ochsenreiter, Torsten*

6F Controlling transferrin receptor trafficking with GPI-valence in bloodstream stage African trypanosomes
Tiengwe, Calvin; *Bush, Peter J.; Bangs, James D.*

TT8 Proteomic analysis of cell-cycle regulation in Trypanosoma brucei
Urbaniak, Michael D.; *Benz, Corinna (Poster #91)*

TT9 Identification of a microtubule-binding kinetochore protein in Trypanosoma brucei
Akiyoshi, Bungo *(Poster #92)*

TT10 RNAi library screening reveals a panel of novel T. b. brucei apolipoprotein-L1 sensitivity determinants, but no role for ICP/cathepsin-L
Alsford, Sam; *Currier, Rachel; Cooper, Anneli; Burrell-Saward, Hollie; MacLeod, Annette (Poster #93)*

TT11 TrypTag: Genome-wide protein localisation in the trypanosome
Sunter, Jack; *Billington, Karen; Halliday, Clare; Madden, Ross; Dean, Samuel Wheeler, Richard (Poster #94)*

SESSION VII: Molecular Biology
Vivian Bellofatto, Chair.
Wednesday 8:45 am

7A Role of long non-coding RNA in Trypanosoma brucei parasite
***Guegan, Fabien**; Bento, Fabio; Neves, Daniel; Manso, Helena; Figueiredo, Luisa*

7B RBP10 is another RNA-binding protein with a key role in the developmental progression to infectivity in Trypanosoma brucei
***Shi, Huafang;** Ramey-Butler, Kiantra; Christiano, Romain; G Kolev, Nikolay; C. Walther, Tobias;Tschudi, Christian*

7C Control of allelic exclusion by a trypanosome 'Vex Histone Chaperone' complex
***Faria, Joana C.;** Glover, Lucy; Hutchinson, Sebastian; Boehm, Cordola; Field, Mark C.; Horn, David*

7D The ZC3H39/40 RNA-binding complex and control of an electron transport chain regulome in African trypanosomes
***Trenaman, Anna;** Hutchinson, Sebastian; Glover, Lucy; Horn, David*

7E Deep analysis of tails on Trypanosoma brucei mitochondrial transcripts reveals life stage as well as transcript-specific regulation
***Zimmer, Sara L.;** Gazestani, Vahid; Hampton, Marshall; Shaw, Aubie; Kalem, Murat; Liggett, Charles; Salavati, Reza*

7F Toward the determination of spindle-associated proteome in Trypanosoma brucei
***Lee, Kyu j.;** Zhou, Qing; Hu, Huiqing; Li, Ziyin*

7G Kinetoplastid Ndc80/Nuf2 reunites chromosome segregation across eukaryotes
***Wickstead, Bill**; D'Archivio, Simon*

POSTERS: Session A
Sunday 7:00 pm

1 FRET Flow Cytometry and Microscopy for Monitoring Glucose in Trypanosoma brucei
Christensen, Kenneth A.; *Voyton, Charles; Ackroyd, Christine; Morris, Meredith; Morris, James*

2 Variant antigen profiling: a tool for genome-wide analysis of Variant Surface Glycoprotein diversity in Trypanosoma congolense
Silva Pereira, Sara; *Casas, Aitor; Haines, Lee; Noyes, Harry; Sanders, Mandy; Berriman, Matthew; Acosta-Serrano, Alvaro; Jackson, Andrew*

3 Trypanosoma brucei RAP1 suppresses VSG switching by repression of telomere transcription and telomere RNA:DNA hybrids
Nanavaty, Vishal P.; *Sandhu, Ranjodh; Jehi, Sanaa; Pandya, Unnati; Li, Bibo*

4 Puzzling together the Trypanosoma brucei Lister 427 genome
Cosentino, Raúl O.; *Müller, Laura SM; Förstner, Konrad U.; Lajoie, Bryan R.; Kaplan, Noam; Belton, Jon-Matthew; Steinbiss, Sascha; Otto, Thomas D.; Berriman, Matthew; Sebra, Robert P.; Dekker, Job; Siegel, T. Nicolai*

5 Biochemical and Genomic Analysis of MRB10130 Function in RNA Editing
Tylec, Brianna*; Simpson, Rachel; Harrison, Gregory; Chen, Runpu; Sun, Yijun; Read, Laurie*

6 A protein kinase inhibitor-based genome-wide RNAi screen to identify regulators of stumpy formation in Trypanosoma brucei
Matthews, Keith R.; *MacDonald, Lindsay; Cayla, Mathieu; Mony, Binny; MacGregor, Paula; Navarro, Miguel*

7 Endosymbiosis, origins and gene expression in the photosynthetic protist Euglena gracilis
Ebenezer, ThankGod*; Nenarokova, Anna; Vanclová, Anna; Zoltner, Martin; Obado, Samuel; Santana, Carlos; Devos, Damien,; Hampl, Vladimir; Ginger, Michael; Lukes, Julius; Dacks, Joel; Carrington, Mark; Kelly, Steven; Field, Mark*

8 RNA Editing Mediator Complex proteins have distinct functions in gRNA utilization and reveal the essentiality of non-contiguous editing modifications
Simpson, Rachel*; Bruno, Andrew; Chen, Runpu; Lott, Kaylen; Tylec, Brianna; Bard, Jonathan; Sun, Yijun; Buck, Michael,; Read, Laurie*

9 The 5S ribonucleoprotein particle (5S RNP) assembly factor protein, Rpf2, is an essential protein in Trypanosoma brucei
Kamina, Anyango*; Williams, Noreen*

10 Flagellum Attachment Zone and the control of Leishmania flagellar pocket shape in both promastigotes and amastigotes
Sunter, Jack D.; *Yanase, Ryuji; Moreira-Leite, Flavia; Gull, Keith*

11 MRB7260 is essential for productive protein-RNA interactions during RNA editing in Trypanosoma brucei
McAdams, Natalie*; Simpson, Rachel; Read, Laurie*

12 Transcriptomes of Trypanosoma brucei rhodesiense from sleeping sickness patients, rodents and culture: effects of growth conditions and RNA preparation methods
Clayton, Christine; *Mulindwa, Julius; Leiss, Kevin; Ibberson, David; Matthews, Keith; Matovu, Enock; Enyaru, John;*

13 Identifying Critical Interactions in the Unique Trypanosoma brucei 5S Ribonucleoprotein Complex and their Role in Ribosome Biogenesis
Jaremko, Daniel*; Williams, Noreen*

14 Exploring regulatory mechanisms behind the function of a major T. brucei arginine methyltransferase
Kafkova, Lucie; *Debler, Erik; Read, Laurie*

15 Characterizing the nuclear export proteins TbMex67-TbMtr2 interactions with the 5S RNP component of the 60S ribosomal subunit in Trypanosoma brucei.
Rink, Constance; *Williams, Noreen*

16 Arginine methylation of TbLpn and its role in phospholipid biosynthesis in Trypanosoma brucei
Spencer, Evan*; Pelletier, Michel*

17 Arginine methylation: a switch that modulates effector functions of DRBD18 in T. brucei
Kaur, Jan Naseer*; Lott, Kaylen; Read, Laurie*

18 Diplonemids and kinetoplastids - diverse, abundant and overlooked components of marine plankton
Lukes, Julius*; Flegontova, Olga; Flegontov, Pavel; Kaur, Binnypreet; Faktorova, Drahomira; Tashyreva, Daria; Prokopchuk, Galina; Malviya, Shruti; Bowler, Chris*

19 The African Trypanosome Evades Immune Clearance by Sugar-Coating its Antigenic Surface
Stebbins, C Erec; *Nesic, Dragana; Pinger, Jason; Ali, Liaqat; Kim, Hee-Sook; Chowdhury, Shanin; Lilic, Mirjana; Cross, George; Ferguson, Michael; Papavasiliou, Nina*

20 Photo-affinity labelling and biochemical analyses identify the target of trypanocidal bis-tetrahydropyran 1,4-triazoles as the FoF1-ATP synthase (mitochondrial complex V)
Tulloch, Lindsay B.; *Menzies, Stefanie K.; Fraser, Andrew L.; Gould, Eoin R.; King, Elizabeth F.; Zacharova, Marija K.; Florence, Gordon J.; Smith, Terry K.*

21 The role of polyamines for proliferation, survival, and infectivity in the protozoan parasite Leishmania donovani
Roberts, Sigrid*; Perdeh, Jasmine; Love, Quintin; Harrelson, John; Boitz, Jan; Yates, Phillip; Ullman, Buddy*

22 Characterisation of an unusual Leishmania HSP70 family member, HRP4
Drini, Sima*; Späth, Gerald; Rachidi, Najma*

23 A genome-scale screen for cell cycle defects in the African trypanosome
Marques, Catarina A.; *Glover, Lucy; Cassidy, Andrew; Horn, David*

24 UMSBP2 is a zinc finger protein essential for chromosome end protection in Trypanosoma brucei
Mishra, Amartya; Klebanov-Akopyan, Olga; Glousker, Galina; Tzfati, Yehuda; Shlomai, Joseph

25 Characterization of the alkyl-dihydroxyacetonephosphate synthase TbADS of Trypanosoma brucei
Zufferey, Rachel*; Lee, Sungsu; Cheung-See-Kit, Melanie; Williams, Tyler*

26 Pentatricopeptide repeat-containing polyadenylation factor KPAF3 defines mitochondrial mRNA identity and stability in trypanosomes
Afasizheva, Inna*; Zhang, Liye; Sement, Francois; Suematsu, Takuma; Yu, Tian*

27 FPC5, an essential, novel and active N-kinesin required for organelle segregation and endomembrane function in Trypanosoma brucei
Perdomo, Doranda*; Berdance, Elodie; Lallinger-Kube, Gertrud; Sahin, Annelise; Landrein, Nicolas; Cayrel, Anne; Ersfeld, Klaus; Kohl, Linda; Robinson, Derrick R.*

28 Trypanosoma brucei RAP1 binds to telomeric DNA in a non-sequence specific manner
Gaurav, Amit K*.; Li, Bibo*

29 Characterising DNA-binding components of the unconventional kinetochore in Trypanosoma brucei
Nerusheva, Olga*; Treep, Joris; Marciano, Gabriele; Akiyoshi, Bungo*

30 Trypanosoma cruzi cell cycle regulation contributes to amastigote plasticity and persistence under stress
***Dumoulin, Peter C.**; Burleigh, Barbara*

31 Ergosterol biosynthesis in Leishmania: protecting parasites in the face of danger?
***Mukherjee, Sumit**; Xu, Wei; Moitra, Samrat; Zhang, Kai*

32 The elucidation of DNA replication monitoring in trypanosomatids allows the obtainment of more accurate values for the duration of cell cycle phases
***da Silva, Marcelo Santos**; Marin Muñoz, Paula Andrea; Elias, Maria Carolina*

33 Functional characterisation of a DYRK kinase implicated in quorum sensing in Trypanosome brucei
***Cayla, Mathieu;** McDonald, Lindsay; MacGregor, Paula; Matthews, Keith R.*

34 A heterodimeric, RNA polymerase II-associated factor is required for SL RNA gene transcription, likely representing the missing transcription factor IIF in trypanosomes
***Srivastava, Ankita**; Badjatia, Nitika; Lee, Ju Huck; Günzl, Arthur*

35 A localisation and functional study of two Leucine Rich Repeat containing proteins and their roles in cell cycle regulation in Trypanosoma brucei brucei.
***Crickley, Robbie**; Mohr, Timm; Vaughan, Sue*

36 Use of Chiral Cell Shape to Ensure Highly Directional Swimming in Trypanosomes
Wheeler, Richard J.

37 Signalling pathway from glucose transporter towards FOF1-ATPase
***Kovarova, Julie;** Horn, David*

38 Trypanosoma cruzi cell death mechanism and physiological changes induced by an organometallic platinum compound
***Mosquillo, Florencia**; Smircich, Pablo; Gambino, Dinorah; Garat, Beatriz; Perez, Leticia*

39 A kinetoplastid-specific alpha-helix-containing protein cooperates with CIF1 to promote cytokinesis initiation in Trypanosoma brucei
***Kurasawa, Yasuhiro**; Zhou, Qing; Li, Ziyin*

40 Molecular dynamics of genome and epigenome ingetrity in Trypanosoma brucei
***Kim, Hee-Sook;** Papavasiliou, Nina*

41 UTRme: a tool to annotate UTRs in Trypanosomatids
Radio, Santiago; *Becco, Lorena; Sotelo, José; Garat, Beatriz; Smircich, Pablo*

42 Dissecting the signaling pathway governing DNA damage-induced G2/M cell cycle checkpoint in Trypanosoma brucei
Zhou, Qing; *Li, Ziyin*

43 Chemical validation of Trypanosma brucei uridine diphosphate-N-acetylglucosamine pyrophosphorylase (UAP) as a drug target
Major, Louise L.; *Cockram, Peter; Ronin, Céline; Lage de Siqueira Neto, Jair; Ciapetti, Paola; McKerrow, James; Smith, Terry*

POSTERS: Session B
Monday 7:00 pm

44 Investigating the linkage between pleomorphism and antigen switch frequency
***McWilliam, Kirsty**; Matthews, Keith*

45 BioID and kDNA segregation proteins in Trypanosoma brucei
***Baudouin, Hélène**; Trikin, Roman; Schimanski, Bernd; Ochsenreiter, Torsten*

46 Genome-wide analysis of mitochondrial translation of Trypanosoma brucei using Ribosome Profiling
***Mesitov, Mikhail**; Yu, Tian; Afasizheva, Inna*

47 Inorganic polyphosphate interacts with nucleolar and glycosomal proteins in trypanosomatids
***S. Negreiros, Raquel;** Lander, Noelia; Smith, Stephanie; H. Morrissey, James; Huang, Guozhong; Docampo, Roberto*

48 RAB11 in the secretory pathway of Trypanosoma brucei
***Umaer, Khan**; Bush, Peter; Bangs, James*

49 The canonical poly(A) polymerase PAP1 polyadenylates non-coding RNAs and is essential for snoRNA biogenesis and the role of snoRNAs in rRNA processing in Trypanosoma brucei
***Michaeli, Shulamit;** Chikne, Vaibhav; Kumar Gupta, Sachin; Doniger, Tirza; Cohen Chalamish, Smadar; Rajan, K. Shanmugha; Waldman Ben-Asher1, Hiba; Unger, Ron; Ullu, Elisabetta; Kolev, Nikolay G.; Tschudi, Christian;*

50 Conserved Motifs in Nuclear Genes Encoding Predicted Mitochondrial Proteins in Trypanosoma cruzi
***Becco, Lorena;** Chavez, Santiago; Smircich, Pablo; Duhagon, María Ana; Garat, Beatriz*

51 Assessing the presence of functional trypanosome lytic factors in human breast milk
***Ebenezer, Karen;** Verdi, Joey ; Raper, Jayne*

52 Elucidating the role of membrane contact sites in calcium signaling pathway of Trypanosoma brucei.
***RAMAKRISHNAN, SRINIVASAN**; Huang, Guozhong; Docampo, Roberto*

53 Lipid rafts and dynamic palmitoylation in protozoan parasite Trypanosoma brucei
Sharma, Aabha

54 A Quorum Sensing-independent Path to Stumpy Development in Trypanosoma brucei
***Zimmermann, Henriette**; Subota, Ines; Kramer, Susanne; Janzen, Christian; Jones, Nicola; Engstler, Markus*

55 Potential new roles for the histone methyltransferase DOT1B in Trypanosoma brucei ?
***Eisenhuth, Nicole**; Butter, Falk; Janzen, Christian J*

56 Direct interaction of TbMORN1 with membrane phospholipids
***Morriswood, Brooke;** Sajko, Sara; Zimmerman, Henriette; Setiawan, Kim; Djinovic-Carugo, Kristina*

57 Cytosolic iron-sulfur assembly pathway interacts with cytosolic ribonucleoprotein granules in Trypanosoma brucei
***Pena-Diaz, Priscila;** Haindrich, Alexander C.; Basu, Somsuvro; Pierik, Antonio J.; Lill, Roland; Lukeš, Julius*

58 Suramin Resistance is associated with a VSG switch in Trypanosoma brucei
***Wiedemar, Natalie**; Wenzler, Tanja; Schmidt, Remo S.; Kunz Renggli, Christina; Cal, Monica; Ndomba, Emiliana; Graf, Fabrice E.; Mäser, Pascal*

59 Target identification for a new series of natural product inspired inhibitors in bloodstream Trypanosoma brucei
***King, Elizabeth**; Gould, Eoin; Fraser, Andrew; Menzies, Stefanie; Zacharova, Marija; Tulloch, Lindsay; Florence, Gordon; Smith, Terry*

60 TbPH1: a kinetoplastid-specific pleckstrin homology domain containing kinesin-like protein
***Kaltenbrunner, Sabine**; Hashimi, Hassan; Lukeš, Julius*

61 Exploiting CRISPR-Cas9 technology to investigate individual histone modifications
***Vásquez Ospina, Juan José**; Wedel, Carolin; Cosentino, Raúl O.; Siegel, T. Nicolai*

62 How do DNA breaks contribute to mosaic VSG formation in Trypanosoma brucei?
***Romero-Meza, Gabriela;** Leitao, Joshua; Mugnier, Monica*

63 Histone acetylation and its associated proteins are major players in defining transcription initiation sites in Trypanosoma brucei
***Kraus, Amelie J.;** Vanselow, Jens T.; ElBashir, Rasha; Wedel, Carolin; Janzen, Christian J.; Förstner, Konrad U.; Schlosser, Andreas; Siegel, T. Nicolai*

64 RNA Editing TUTase 1: structural foundation of substrate recognition, complex interactions and drug targeting
***Suematsu, Takuma**; Rajappa-Titu, Lional; Munoz-Tello, Paola; Long, Marius; Demir, Özlem; Cheng, Kevin J; Stagno, Jason R.; Luecke, Hartmut; Amaro, Rommie E; Afasizheva, Inna; Thore, Stéphane; Afasizhev, Ruslan*

65 The translationally controlled tumor protein (TCTP) in T. brucei
***Jojic, Borka;** Amodeo, Simona; Ochsenreiter, Torsten*

66 Investigating the function of Translesion DNA Polymerase Nu (PolN) in Trypanosoma brucei
***Zurita-Leal, Andrea**; Lemgruber, Leandro; McCulloch, Richard*

67 The targeting complex of the cytoplasmic FeS cluster assembly machinery is essential in Trypanosoma brucei
***Tonini, Maiko**; Smith, Terry; MacNeill, Stuart*

68 Characterization of heterochromatin formation in Trypanosoma brucei brucei
***Carloni, Roberta**; Staneva, Desislava; Matthews, Keith; Allshire, Robin*

69 R loops: DNA:RNA hybrids with critical roles in the genome biology of Trypanosoma brucei
***Briggs, Emma**; Lemgruber, Leandro; Crouch, Kathryn; McCulloch, Richard*

70 Characterisation of three independent T. brucei 'double-expressor' strains showing simultaneous activation of two VSG expression sites
***Kerry, Louise**; Davies, Carys; Budzak, James; Pegg, Elaine; Witmer, Kathrin; Hall, Belinda; Kushwaha, Manish; D'Archivio, Simon; Wickstead, Bill; Rudenko, Gloria*

71 Genome-wide mutagenesis and multi-drug resistance in Trypanosoma cruzi induced by benznidazole
***Kelly, John;** Campos, Monica; Phelan, Jody; Francisco, Amanda; Taylor, Martin; Lewis, Michael; Pain, Arnab; Clark, Taane*

72 The damage signalling kinase ATR plays a role in Trypanosoma brucei genome maintenance and antigenic variation
***Stortz, Jennifer Ann;** Lemgruber-Soares, Leandro; Mottram, Jeremy C; McCulloch, Richard*

73 High frequency of RNA leishmania-virus associated with clinical samples of mucosal leishmaniasis caused by L. braziliensis but not L. panamensis
***Echeverry, Maria;** Parra-Muñoz, Diana Marcela; Ovalle-Bracho, Clemencia*

74 Intrinsic DNA curvature in trypanosomes
***Smircich, Pablo;** El-Sayed, Najib M.; Garat, Beatriz*

75 Application of a T. brucei whole-genome gain-of-function library toward identification of genes that promote drug resistance: methodological development and preliminary findings
***Quinn, Mckenzie**; Gomez, Stephanie; Schulz, Danae; Hovel-Miner, Galadriel*

76 Completion of a Trypanosoma brucei whole-genome gain-of-function library
***Gomez, Stephanie;** Schulz, Danae; Kim, Hee-sook; Papavasiliou, Nina; Hovel-Miner, Galadriel*

77 Chemical genetics to study the function of deubiquitinases in Leishmania differentiation.
***Damianou, Andreas**; Mottram, Jeremy; Rodenko, Boris; Burchmore, Richard; Celie, Patrick*

78 Protein networks link base J to RNAPII-mediated transcription
***Jensen, Bryan C.;** Gillespie, Mark; Phan, Isabelle; Heidebrecht, Tatjana; Perrakis, Anastassis; Ranish, Jeffrey; Myler, Peter*

79 Compiling a minicircle genome for Trypanosoma brucei
***Yu, Tian**; Zhuang, Liye; Monti, Stefano; Afasizhev, Ruslan*

80 Processing and targeting of Cathepsin L (TbCatL) to the Lysosome in Trypanosoma brucei
***Koeller, Carolina M.;** Bangs, James D.*

81 Trypanosoma cruzi infection alters host central carbon metabolism and increases glucose uptake for potential parasite utilization
***Shah-Simpson, Sheena;** Lentini, Gaelle; Dumoulin, Peter; Burleigh, Barbara*

82 Reconstitution and purification approaches to study architecture of multiprotein RNA editing complexes in trypanosomes
***Wang, Hong;** Afasizheva, Inna; Afasizhev, Ruslan*

83 In vivo cleavage specificity of Trypanosoma brucei editosome endonucleases
***Carnes, Jason;** McDermott, Suzanne; Anupama, Atashi; Oliver, Brian G.; Sather, D. Noah; Stuart, Kenneth*

84 Outer-kinetochore phosphatase function suggests a conserved mode of attachment regulation in trypanosomes
***Brusini, Lorenzo**; Wickstead, Bill*

85 Probing the nutritional requirements and essential metabolic functions of the trypanosomatid lysosome
***Young, Simon A.;** Smith, Terry*

86 The ciliary gate: how are intraflagellar transport proteins concentrated at the flagellum base?

Jung, Jamin*; Santi-Rocca, Julien; Fort, Cècile; Perrot, Sylvie; Bastin, Philippe*

87 Physicochemical optimization of lapatinib analog NEU-1953 for human African trypanosomiasis drug development

Bachovchin, Kelly; *Ferrins, Lori; Klug, Dana; Bag, Seema; Singh, Baljinder; Tanghe, Scott; Rodriguez, Ana; Thomas, Sarah M.; Wiedman, Justin; Sharma, Amrita; Mensa-Wilmot, Kojo; Sciotti, Richard J.; Pollastri, Michael P.*

POSTERS: Session C
Tuesday 7:00 pm

88 Mutagenesis screens identify editosome protein domains that differentially affect RNA editing between life cycle stages of Trypanosoma brucei
McDermott, Suzanne M.; *Carnes, Jason; Stuart, Kenneth*

89 RNA-binding protein capture reveals a diversity of poly(A)+ RNA-binding proteins in insect-form Trypanosoma brucei
Fisher, Michael; *Das, Anish; Bellofatto, Vivian*

90 High-efficiency chemical-mediated transfection of Trypanosoma cruzi
Olmo, Francisco; *Rotger, Carmen; Taylor, Martin C; Costa, Fernanda C; Costa, Antonio; Kelly, John M*

91 Proteomic analysis of cell-cycle regulation in Trypanosoma brucei
Urbaniak, Michael D.; *Benz, Corinna*

92 Identification of a microtubule-binding kinetochore protein in Trypanosoma brucei
Akiyoshi, Bungo

93 RNAi library screening reveals a panel of novel T. b. brucei apolipoprotein-L1 sensitivity determinants, but no role for ICP/cathepsin-L
Alsford, Sam; *Currier, Rachel; Cooper, Anneli; Burrell-Saward, Hollie; MacLeod, Annette*

94 TrypTag: Genome-wide protein localisation in the trypanosome
Sunter, Jack; *Billington, Karen; Halliday, Clare; Madden, Ross; Dean, Samuel; Wheeler, Richard*

95 Lipid rafts and dynamic palmitoylation in protozoan parasite Trypanosoma brucei
Sharma, Aabha

96 Metacyclogenesis of Leishmania is halted in the absence of proline alanine transporter
Zilberstein, Dan;*Schlisselberg, Doreen; Koren Nitzan, Roni; Iankovici, Liat; Inbar, Ehud*

97 Interaction of the Trypanosoma brucei cap-binding protein EIF4E2 with a histone-mRNA binding protein homolog
de Melo Neto, Osvaldo P.; *Freire, Eden; Bezerra, Maria Jose; Vashisht, Ajay; Zamudio, Jesse; Sharp, Philip; Rezende, Antonio Mauro; Wohlschlegel, James; Sturm, Nancy; Campbell, David;*

98 The Divergent Small Tim Homologues are Essential for Import and Assembly of TbTim17 and Other Polytopic Mitochondrial Inner Membrane Proteins in Trypanosoma brucei
Smith, Joseph T.; *Singha, Ujjal; Misra, Smita; Chaudhuri, Minu*

99 New insights into the biosynthetic pathway of VSGs in Trypanosoma brucei
Jones, Nicola G.; *Reimer, Anastasija; Stigloher, Christian; Engstler, Markus*

100 Two Novel Components of Trypanosoma brucei Mitochondrial Inner Membrane Translocase, Tim62 and Tim54, Possess Different Substrate Specificities
Singha, Ujjal

101 Molecular Characterization of Trypanosoma cruzi TcVps26-Like
Rubio Ortiz, Margarita*; Martínez Calvillo, Santiago; Manning Cela, Rebeca Georgina*

102 Comparative studies of the T. brucei malate dehydrogenase isoforms
Springer, Amy L.

103 Essential or redundant? Dissection of flagellar pocket protein function in African trypanosomes
Whipple, Sarah*; Gadelha, Catarina*

104 Some peculiarities of kinetoplastid lipid metabolism
SMITH, TERRY; *YOUNG, SIMON; MAJOR, LOUISE; DENTON, HELEN; ROBERTS, MATTHEW; CORON, ROSS; DICKIE, EMILY*

105 Mapping of the binding sites of naphthalene-based inhibitors on Trypanosoma brucei RNA editing ligase 1
Mehta, Vaibhav*; Moshiri, Houtan; Salavati, Reza*

106 Lipidomics analysis of the intracellular human parasite, Trypanosoma cruzi, reveals potential sources of lipid scavenging from host cells.
Gazos-Lopes, Felipe*; Martin, Jessica; Burleigh, Barbara*

107 The role of de novo myo-inositol synthesis and metabolism in Leishmania parasites
Liu, Tim Chung-Ting*; Sansom, Fiona; McConville, Malcolm*

108 The trypanosome outer kinetochore complex
D Archivio, Simon*; McDonald, Jennifer; Wickstead, Bill*

109 The trypanosome flagellum transition zone is a bidirectional conduit that operates in the absence of the flagellar pocket
Dean, Samuel; *Gull, Keith*

110 Different classes of RNA require distinct Mex67 paralogs for processing and nucleocytoplasmic export in trypanosomes.
Obado, Samson*; Zhang, Wenzhu; Brillantes, Marc; Field, Mark; Chait, Brian; Rout, Michael*

111 Expression site associated genes are not essential for Trypanosoma brucei survival in vitro
***Chamberlain, James**; Gadelha, Catarina*

112 Targeted Approach for Trypanomiasis Theraphy : Hexokinase in the Crosshair
Suryadi, Jimmy

113 Molecular architecture of the axoneme of Trypanosoma brucei by cryo-electron tomography
Imhof, Simon

114 Involvement of TcAlba30 in the stage-specific regulation of ß-amastin expression in Trypanosoma cruzi
***Perez Diaz, Leticia**; Smircich, Pablo; Silva, Tais; Teixeira, Santuza*

115 Exploring the bioenergetics of the bloodstream T. brucei mitochondrion
***Panicucci, Brian P.;** Taleva, Gergana; Ielanskyi, Mykyta; Zikova, Alena*

116 Blood stream form Trypanosoma brucei regulates intracellular glucose levels in a density dependent manner
***Qiu, Yijian;** Voyton, Charles; Christensen, Kenneth; Morris, James*

117 Cytoplasmic RNA uridyltransferase TbTUT4 is essential in monomorphic bloodstream form of Trypanosoma brucei.
***Knusel, Sebastian;** Zhang, Liye; Qian, Yu; Costello, Catherine; Monti, Stefano; Roditi, Isabel; Afasizhev, Ruslan*

118 Structural basis of prozyme activation of the major protein arginine methyltransferase in Trypanosoma brucei
***Debler, Erik W.;** Kafkova, Lucie; Read, Laurie K.*

119 Phospholipase A2 of Trypanosoma cruzi: in silico analysis and three-dimensional structure modeling of the protein
***Soares Coelho, Felipe**; Monteiro Torres, Pedro; Silva, Manuela; Bisch, Paulo; Pascutti, Pedro; Lopes, Angela H.*

120 Transcriptomic and Translatomic landscape of the Trypanosoma cruzi cell cycle.
***Duhagon, Maria A.;** Chavez, Santiago; Eastman, Guillermo; Smircich, Pablo; Garat, Beatriz; Sotelo-Silveira, José Roberto*

121 Understanding the TLF mediated killing of Leishmania sp.
***Pant, Jyoti**; Keceli, Mert; Nelson, Maria; Raper, Jayne*

122 Gorilla apolipoprotein L-1 lyses T. b. brucei and is associated with both TLF1 and TLF2 in serum.
Gonzalez Baradat, Bernardo; *Rosa, Alessandro; Raper, Jayne; Verdi, Joey; Ebenezer, Karen; Weiselberg, Jessica; Hodosh, Shana*

123Post-transcriptional regulation of purine transporters in the Leishmania donovani purine stress response
Yates, Phillip A.; *Licon, Haley; Martin, Jessica; Ortiz, Diana; Soysa, Radika; Vanderwerf, Scott; Ullman, Buddy; Landfear, Scott; Carter, Nicola*

124 Inhibitor Screening against Trypanosoma cruzi RNA Triphosphatase
Takagi, Yuko*; Kuwabara, Naoyuki; Ho, Kiong; Sakashita, Hitoshi; Furukawa, Koji*

125 Functional analysis of TOEFAZ1 uncovers protein domains essential for cytokinesis in Trypanosoma brucei
de Graffenried, Christopher L.; *Sinclair-Davis, Amy; McAllaster, Michael*

126 The role of rapid mitochondrial dynamics in kinetoplastid parasites
Povelones, Megan L.; *Ruthel, Gordon; DiMaio, John*

127 The glycobiology of African trypanosomes upon adaptation to living in adipose tissue
Rodrigues, Joao*; Wongtrakul-Kish, Katherine; Trindade, Sandra; Pinto-Neves, Daniel; Spencer, Daniel; Figueiredo, Luisa*

128 Crithidia fasciculata as a model to study insect-specific adhesion
Povelones, Michael; *Filosa, John; Dudkin, Elizabeth; Povelones, Megan*

129 TriTrypDB: The Functional Genomics Resource for Kinetoplastids
Warrenfeltz, Susanne; *Crouch, Kathryn; Hertz-Fowler, Christiane; ...on behalf of the, Kinetoplastid Database Consortium*

130 Role of the RNA binding protein RBP42 in Trypanosoma brucei bloodstream form parasites
Das, Anish

131 The homologue of the yeast SNARE protein Ykt6 functions in glycosome assembly in Trypanosoma brucei
Banerjee, Hiren*; Rachubinski, Richard A.*

SESSION I: Cell Biology I
Scott Landfear, Chair.
Saturday 7:00 pm

04/22/17

1A From solitary swimmers to swarms and back - trypanosomes on their journey through the tsetse fly

Schuster, Sarah (Universität Würzburg, USA); Krüger, Tim (Universität Würzburg, USA); Subota, Ines (Universität Würzburg, USA); Thusek, Sina (Universitätsklinikum Würzburg, USA); Rotureau, Brice (Institut Pasteur, USA); Kollmannsberger, Philip (Universität Würzburg, USA); Beilhack, Andreas (Universitätsklinikum Würzburg, USA); Engstler, Markus (Universität Würzburg, USA)

Trypanosoma brucei undergoes a complex life cycle in the tsetse fly vector. The parasite´s development occurs during a journey through the different microenvironments of the fly´s internal organs. For the trypanosomes this involves crossing various barriers, confined surroundings, and swimming against flow and peristaltic movement. Additionally, they undergo major and drastic morphological changes. The parasite´s motility, which is directly dependent on morphology, is essential for its survival and successful development. This work details cell morphology, motility, and collective behaviour of different developmental stages from the tsetse fly using high spatiotemporal resolution microscopy. Using fluorescently-labelled parasites, swimming patterns of solitary swimmers were analysed *in vivo* and *in vitro*, as well as collective motion at the single cell level *in vivo*. We show that trypanosomes are able to synchronise their flagellar beats and produce superordinate wave patterns at high cell concentrations, probably by hydrodynamic self-organisation inside the fly interstices. Additionally, light sheet fluorescence microscopy was established as a powerful tool for the 3D-analysis of the infection process in the tsetse fly´s digestive tract. The results provide information about tissue geometry and topology with unprecedented resolution. We were able to visualise fluorescent trypanosomes inside the surprisingly complex folds of the peritrophic matrix at a single cell level. Calculations using 3D Euclidean distance mapping allows us to measure the void space for trypanosomes to navigate within this labyrinth. In summary, we provide a detailed view on trypanosome motile behaviour as a function of development in diverse host surroundings. We propose that the infection process is an alternating succession of solitary and collective motions, adapted to the varying and convoluted microenvironments inside the vector.

1B Swim like your lifecycle depends on it: The impact of Leishmania flagellar motility

Findlay, Rachel (Centre for Immun. and Infection, Depts Biology and Physics, Univ. of York, USA); Gadelha, Hermes (Dept of Mathematics, Univ. of York, USA); Rogers, Matthew (London Sch Trop Hyg and Medicine, USA); Wilson, Laurence (Dept of Physics, Univ. of York, USA); Walrad, Pegine B. (Centre for Immun. and Infection, Univ. of York, University of York, GBR)

Motility of *Leishmania* parasites is essential for survival during host transitions and is important for lifecycle progression. It is the presence of an appendage, at the anterior end of the promastigotes which allows parasite movement. This oscillating flagellum creates the force which enables the promastigote to be pulled through its environment. The environmental conditions of the parasite change significantly during the lifecycle and the parasite morphologically transforms to optimise its infection potential. The details of parasite swimming behaviour remain obscure but are fundamentally linked to its infectivity. Our investigation focuses upon how flagellar structure adapts between promastigote forms and how this impacts function in distinct environmental conditions.

We have adapted a unique method of high-speed, three-dimensional imaging called digital inline holographic microscopy (DIHM) allowing us to examine the movements of *Leishmania mexicana* promastigotes. The data produced can be used to numerically refocus and create a high resolution reconstruction of parasite movement at different length scales. Using stage-specific fluorescent markers to distinguish between the promastigote stages, we tracked parasites over multiple frames to gain information on the swimming patterns of these cells. Tracking of promastigotes demonstrates stage-specific differences in swimming behaviour. At a higher magnification, the shape of an individual flagellum can be reconstructed allowing in depth three-dimensional analysis and comparison of flagellum structural modification throughout its lifecycle. DIHM has allowed us to mathematically quantify the relationship between the active bending of the flagellum and cell movements within contrasting environments. We have elucidated distinct characteristics in the flagellar structures of promastigotes and these distinctions promote parasite transmission.

1C Discovery of new Leishmania motility mutants in a high-throughput CRISPR-Cas9 knockout screen of the flagellar proteome

Beneke, Tom (University of Oxford, Sir William Dunn School of Pathology, Oxford, GBR); Madden, Ross (University of Oxford, Sir William Dunn School of Pathology, USA); Demay, Francois (University of Oxford, Sir William Dunn School of Pathology, USA); Valli, Jessica (University of Oxford, Sir William Dunn School of Pathology, USA); Wheeler, Richard (University of Oxford, Sir William Dunn School of Pathology, USA); Sunter, Jack (University of Oxford, Sir William Dunn School of Pathology, USA); Gluenz, Eva (University of Oxford, Sir William Dunn School of Pathology, USA)

The *Leishmania* flagellum is a multifunctional organelle used for motility, attachment to the sand fly vector and sensory functions. To discover the flagellar protein composition and enable a dissection of different flagellar functions, we developed a new deflagellation protocol for *L. mexicana* promastigotes. Separated flagella and deflagellated cell bodies were analysed by mass spectrometry. Using a label-free quantification method, 620 flagellar and 1,871 cell body enriched proteins were identified. To enable analysis of large-'omics' datasets such as this, we developed a CRISPR-Cas9 gene editing method to dissect function and subcellular localisation of hundreds of proteins in a high-throughput manner, allowing gene editing in a range of kinetoplastids. Donor DNA and sgRNA templates are produced simply by PCR without the need for time-consuming DNA cloning and we developed an online resource LeishGEdit.net for the design of CRISPR primers. We used this method to fluorescently tag 90 *L. mexicana* proteins identified in the flagellar proteome; 78 proteins localised to the flagellum, identifying novel components of the flagellar axoneme, PFR, matrix and flagellar membrane. 43 of these 78 genes were then targeted for knockout using CRISPR-Cas9, resulting in 24 viable knockout cell lines. Six of the mutants had severe phenotypes, including impaired motility and flagellar ultrastructural defects. Our data shows that the *L. mexicana* flagellar proteome defined in this study is a rich dataset that will facilitate further dissection of flagellar function in *Leishmania* spp. Moreover, we show how our gene editing method enables screening of pooled populations of barcoded mutants for the discovery of mutant phenotypes in *Leishmania* spp. and trypanosomes.

1D Molecular and systems analysis of cell-cell communication and social behavior in Trypanosoma brucei

DeMarco, Stephanie (Molecular Biology Interdepartmental Doctoral Program, University of California, Los Angeles, Los Angeles, CA, USA); Stream, Alexandra (Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, USA); Jahanbakhsh, Shahriyar (Department of Chemistry and Biochemistry, University of California, Los Angeles, USA); Hill, Kent (Molecular Biology Institute, University of California, Los Angeles, USA)

During transmission through the tsetse fly, *Trypanosoma brucei* lives and grows in constant contact with fly tissue surfaces. In bacteria, surface growth profoundly influences bacterial physiology, pathogenesis and behavior, including promoting social behaviors such as biofilm formation and social motility. Likewise, when cultivated on surfaces, fly midgut-stage *T. brucei* parasites assemble into multicellular groups that can sense and respond collectively to extracellular signals. This behavior, termed social motility (SoMo), is developmentally regulated, and SoMo defects correlate with decreased fly infection [1]. While surface-associated group behaviors have been well-studied in bacteria, the genes and signaling systems that underlie social behaviors in protozoan pathogens are mostly unknown. SoMo relies on cAMP regulatory systems in the *T. brucei* flagellum, specifically members of the adenylate cyclase (AC) family and cAMP-specific phosphodiesterase (PDEB1) [2]. When a subset of AC proteins are knocked down, *T. brucei* exhibits a hypersocial phenotype, yet when PDEB1 is lost, *T. brucei* can no longer do SoMo. Because *T. brucei* has to sense its environment and enact a particular signaling cascade to behave socially, SoMo presents a novel assay to study signaling in these parasites, an important yet understudied aspect of trypanosome biology. To further define gene expression programs that control social motility, we have utilized transcriptomics to compare gene expression profiles of parasites engaging in SoMo versus planktonic cells in suspension culture, as well as wildtype parasites versus *somo*- PDEB1 mutants. Our results reveal large-scale changes in gene expression connected with the transition from a planktonic lifestyle to a surface-associated parasite community. As parasites advance outward from the initial site of surface colonization expression of hundreds of genes is further altered, indicating substantial developmental changes in parasite physiology. RNAi against differentially regulated genes was used to assess their requirement for SoMo and identified new candidate SoMo genes. This work offers insight into genes directing parasite social behavior and parasite cell-cell signaling.

[1] Imhof and Roditi, *Trends in Parasitology*, 2015.

[2] Saada, DeMarco, Shimogawa, and Hill, *PLoS Pathogens*, 2015.

1E Inositol phosphates regulation of Trypanosoma brucei life cycle developmental progression

Cestari, Igor (Center for Infectious Disease Research, Seattle, WA, USA); Anupama, Atashi (Center for Infectious Disease Research, USA); Stuart, Ken (Center for Infectious Disease Research, USA)

Developmental progression through *Trypanosoma brucei* life cycle involves regulation of stage-specific surface proteins, morphological and metabolic changes to adapt to physiological conditions in the mammalian host and tse-tse fly vector. In mammals *T. brucei* circulates as replicative slender bloodstream forms (BFs) which differentiate into stumpy non-dividing BFs. Once taken by the fly stumpy BFs differentiate into replicative procyclic forms (PFs). We found that the inositol phosphate pathway plays a role in regulating *T. brucei* development. Conditional knockdown of inositol polyphosphate 5-phosphatase (IP5Pase), which is predicted to dephosphorylate the 5-phosphate of Ins(1,4,5)P3, resulted in cell density dependent slender to stumpy differentiation. Moreover, knockdown of inositol polyphosphate multikinase (IPMK), which we show to phosphorylates Ins(1,4,5)P3 at 3 and 6 positions, resulted in >10-fold increase (~40%) in BF to PF differentiation as determined by morphology, loss of VSGs and procyclin expression. RNAseq analysis after 36h of IPMK knockdown in BFs showed upregulation of BF and PF stage-specific gene sets, which includes sugar and amino acid transporters, cAMP signaling, oxidative phosphorylation (oxphos), and also resulted in a distinct pattern of RNA-binding protein (RBP) expression. Overexpression of RBP6, RBP7A or PPCT1 altered expression of BF and PF stage-specific gene sets, but did not increase differentiation, which imply a role for RBPs in controlling developmentally regulated mRNA expression. Affinity purification and mass spectrometry analysis of proteins interacting with IPMK substrate and product revealed that Ins(1,4,5)P3 was enriched with proteins that function in signaling, protein synthesis and degradation whereas Ins(1,3,4,5)P4 was enriched with proteins of energy metabolism. IPMK or IP5Pase knockdown altered BFs metabolism resulting in ~2.5-fold increase in ATP production and a decrease in pyruvate released, respectively. Importantly, the excess of ATP production after IPMK knockdown in BFs was inhibited by oxphos inhibitors, which suggest that IPMK regulates the metabolic switch between glycolysis and oxphos (and hence RNA editing) during development. The IPMK knockdown increased *T. brucei* sensitivity to citrate/cis-aconitate induced differentiation, and differentiation rates were decreased by oxphos inhibition. The data imply that inositol phosphates are part of a regulatory network that controls *T. brucei* metabolism and life cycle development.

1F A protein kinase A (PKA) signaling pathway mediates promastigote-to-amastigote differentiation in Leishmania

Zilberstein, Dan (Technion-Israel Institute of Technology, Haifa, ISR); Fischer Weinberger, Renana (Technion-Israel Institute of Technology, USA); Koren Nitzan, Roni (Technion-Israel Institute of Technology, USA); Bar Avraham, Yael (Technion-Israel Institute of Technology, USA); Myler, Peter, J. (2Center for Infectious Disease Research, USA)

Protozoan parasites of the genus *Leishmania* cycle between promastigotes in the sand fly mid-gut and amastigotes in the phagolysosome of mammalian macrophages. While much of the molecular mechanism of development inside macrophages remains a mystery, development of a host-free system that simulates phagolysosome conditions (37ºC and pH 5.5) has provided new insight into these processes. Using high coverage transcriptomic and proteomic approaches to interrogate changes in gene expression during promastigote-to-amastigote differentiation of *Leishmania donovani* have shown that differentiation is well-regulated, with ordered and coordinated changes in mRNA and protein abundance resulting in the parasites re-tooling their metabolic pathways for life in the new host environment. Phosphoproteomic analyses also revealed substantial changes in protein phosphorylation within minutes of exposure to the differentiation signal. Null mutants of early-activated protein kinases revealed that protein kinase A (PKA)-mediated signaling pathway plays a critical role in initiating the transition between lifecycle stages. PKA is ubiquitous in eukaryotic cells. Activity of the catalytic subunit(s) (PKAC) is controlled by assembly with the regulatory subunit(s) (PKAR) into holoenzyme complex that is inactive in the absence of cAMP. Cyclic nucleotide binding domains (cNBDs) in the PKAR bind cAMP, resulting in a conformational change, dissociation of the PKAC-PKAR complex and activation of PKAC. Our analysis indicated that, in *L. donovani*, changes in the interaction between PKAC3 and PKAR′ are crucial for the response to the temperature and pH signals, as well as the timing of the differentiation process. Null mutants of PKAR' differentiate at a rate slower than wild type and die during the last phase of differentiation. Time-course analysis of PKAC3 phosphorylation during phase I supports a critical role in *L. donovani* promastigote-to-amastigote differentiation. Overall, our work provides the first insight into the molecular mechanism of *Leishmania* differentiation by identifying the signaling pathway that initiates this process.

1G Conservation of quorum-sensing signal responses and cross-species interactions between Trypanosoma brucei and Trypanosoma congolense

Silvester, Eleanor (University of Edinburgh, USA); Matthews, Keith (University of Edinburgh, USA)

Trypanosoma brucei exhibits quorum-sensing (QS) to control its growth and development in the bloodstream. As replicating slender forms elevate parasite numbers, parasite-derived 'stumpy induction factor' accumulates, driving differentiation to cell-cycle arrested 'stumpy forms'. Stumpy forms are competent for transmission by the tsetse fly vector, such that the production of stumpy forms can impact parasite virulence and transmission.

Although QS and its underlying molecular processes have been studied in *T. brucei*, co-infections involving both *T. brucei* and *Trypanosoma congolense* are common in the field. Therefore, we have investigated whether the capacity for QS is conserved in *T. congolense* and whether cross-talk can occur between these two species in co-infections. Firstly, we have found that *T. congolense* does exhibit density-dependent growth control *in vivo*, with an accumulation of G1-arrested cells coinciding with peak parasitaemia. Secondly, from *T. congolense* genome analysis, we have identified many orthologues of *T. brucei* QS-regulatory genes and demonstrated the ability of one to functionally complement, restoring differentiation capacity to a *T. brucei* null mutant. Thirdly, using an engineered *T. brucei* pleomorphic line that quantitatively reports on stumpy formation, we have shown that *T. congolense*-conditioned culture medium inhibits *T. brucei* growth and activates stumpy reporter expression *in vitro*. This effect was diminished upon RNAi-silencing a *T. brucei* QS gene, thereby linking the *T. congolense*-induced effect to the *T. brucei* QS response pathway. Finally, we have shown that *T. congolense* accelerates *T. brucei* differentiation to stumpy forms in coinfections, demonstrating inter-species interactions between *T. congolense* and *T. brucei in vivo*. These interactions have important implications for trypanosome virulence, transmission, competition and evolution in the field.

1H Advances in the identification of the stumpy induction factor (SIF)

Subota, Ines (Universität Würzburg, USA); Fekete, Agnes (Universität Würzburg, USA); Müller, Martin J (Universität Würzburg, USA); Engstler, Markus (Universität Würzburg, USA)

Trypanosoma brucei parasites efficiently proliferate in the mammalian host and would rapidly kill it without a quorum sensing-based growth control system. The main player herein is the stumpy induction factor (SIF) which is secreted by the parasite during growth. When its concentration exceeds a certain level, meaning that a critical cell density is reached, differentiation is initiated. Formerly proliferating slender forms become stumpy in morphology and arrest their cell cycle in G1/G0. Concomitant changes in metabolism and other adaptations make stumpy cells suited for infecting the tsetse fly host.

The existence of SIF has been proposed two decades ago and this factor could be an appealing drug lead against trypanosomiasis, however, has to date eluded to reveal its molecular nature. With the combination of different techniques of liquid chromatography (LC) and untargeted metabolomics we aim at a stepwise isolation of SIF. The right path to choose is determined by the detection of SIF activity, relating reduced cell growth with the expression of a PAD1 reporter.

Conditioned medium was harvested and the protein content extracted by methanol precipitation. The resulting protein-free concentrate has been further purified by reversed-phase solid phase extraction. The highly polar nature of SIF became apparent when it eluted with 10% methanol in water, which further implied the use of hydrophilic interaction LC using an amide column. The SIF activity was determined in only a small number of fractions comprising less than 100 metabolites each. Surprisingly, the active fractions contained pyruvate and related alpha-keto-acid compounds that are currently investigated and subjected to further purification steps. We present data that finally allow speculation on the molecular nature of SIF.

SESSION II: Host-Parasite Interactions
Sam Alsford, Chair.
Sunday 8:45 am
04/23/17

2A Dissecting Leishmania metabolism and population heterogenity in infected tissues

Saunders, Eleanor (University of Melbourne, USA); Kloehn, Joachim (University of Melbourne, USA); Sernee, Fleur (University of Melbourne, USA); Ralton, Julie (University of Melbourne, USA); McConville, Malcolm (University of Melbourne, USA)

Leishmania amastigotes target macrophages and other phagocytic cells in the mammalian host and are responsible for sustaining both acute and chronic stages of infection. Information on the metabolism and physiological state of this stage is crucial for understanding how these parasites persist long term in the mammalian host and for identifying new drug targets. Using a combination of advanced metabolite profiling, ^{13}C-stable isotope labeling and genetic approaches we have shown that amastigotes are dependent on the utilization of sugars and amino-sugars salvaged from the macrophage phagolysosome. Interestingly, *L.mexicana* lines deficient in the uptake of sugars can switch to a highly efficient gluconeogenic mode and survive in ex vivo infected macrophages, but not in susceptible mice, highlighting the importance of hexose metabolism for virulence *in vivo*. Complementary heavy water ($^{2}H_2O$) labeling studies in infected mice has shown that lesion amastigotes grow very slowly, confirming *in vitro* studies suggesting that these stages switch to a metabolically quiescent state (stringent response). In more recent studies we have used to high resolution MALDI-imaging mass spectrometry to measure amastigote metabolic heterogeneity in lesions. We show that lesion amastigotes are quite heterogeneous, with subpopulations distributed throughout the lesion that exhibit very slow rates of growth. These hyper-quiescent populations may contribute to the long term persistence and intrinsic drug resistance of lesion amastigotes.

2B TcMcS-Parasite Pressure Gauge: Regulatory role of a mechanosensitive channel in T. cruzi physiological mechanisms

Dave, Noopur (1Center for Applied Biotechnology Studies and Department of Biological Science, Natural Sciences and Mathematics, California State University, Fullert, USA); Barerra, Patricia (1Center for Applied Biotechnology Studies and Department of Biological Science, Natural Sciences and Mathematics, California State University, Fullert, USA); Cetiner, Ugur (2Department of Biology, University of Maryland, College Park, MD, USA); Sukharev, Sergei (2Department of Biology, University of Maryland, College Park, MD, USA); Jimenez, Veronica (1Center for Applied Biotechnology Studies and Department of Biological Science, Natural Sciences and Mathematics, California State University, Fullert, USA)

Trypanosoma cruzi faces various environmental challenges during its life cycle as it propagates from an insect vector to a mammalian host during its life cycle. To cope with these changes, *T. cruzi* has developed robust compensatory mechanisms; however, the sensory machinery utilized to detect variations in the extracellular conditions remains unknown. In all cell types, mechanosensation is responsible for sensing and responding to changes in pressure, osmolarity, and tension of the membrane. In addition, in bacteria mechanosensation is associated with increase in biofilm formation, quorum sensing and activation of virulence-related genes. In *T. cruzi*, we have identified and characterized a mechanosensitive channel (TcMcS) that shares structural and functional traits with the small conductance mechanosensitive channel, MscS of *E. coli*. The presence of homologues suggests a conserved mechanism for osmoadaptation in other protozoan parasites.

Single-channel electrophysiological characterization of TcMcS in *E. coli* spheroplast showed a tension-dependent activation within the expected range for MscS channels. A linear non-rectifying conductance of 0.4 nS suggests a pore that is permeable to ions, amino acids and compatible osmolytes that can be transported across membranes in the course of adaptation.

Immunofluorescence studies show that TcMcS is differentially localized in the three main life stages of *T. cruzi* with localization on the contractile vacuole and the plasma membrane. Gene targeting by CRISPR-Cas9 caused severe defects in morphology, impaired growth and decreased infectivity of the parasites. TcMcS knockdown mutants showed major reduction in metacyclogenesis and intracellular replication.

Under hyposmotic stress, cells overexpressing TcMcS swell less while knockout mutants swell significantly more and are not able to recover their normal volume. Known mechanosensitive channel blockers like gadolinium, cause significant differences in the parasite's ability to detect and compensate its volume under osmotic stress.

Overall, our results support the physiological role of TcMcS in *T. cruzi* osmoregulation and infectivity.

Funding: CSUPERB Eden Award, CSUPERB NI Grant, AHA 16GRNT30280014, NIH R15AI122153.

2C Tracking single Trypanosoma cruzi parasites to reservoir sites in the gut during chronic murine infections

Taylor, Martin C. (London School of Hygiene and Tropical Medicine, London, GBR); Fortes-Francisco, Amanda (USA); Ward, Alexander (USA); Jayawardhana, Shiromani (USA); Lewis, Michael (USA); Kelly, John (USA)

Trypanosoma cruzi causes a chronic life-long infection. Parasites are rarely observed in the blood and infected patients may routinely be PCR-negative. Highly sensitive bioluminescence imaging (BLI) has allowed us to establish the GI tract as the major reservoir site in murine models of chronic *T. cruzi* infections. However, BLI has limitations in its ability to identify parasites at a cellular level, and to characterise the local immunological environment. To circumvent this, we generated trypanosomes expressing a luciferase-mNeonGreen fusion protein, such that parasites were both highly bioluminescent and fluorescent. In acutely infected mice, we observed "amastigote nests" in the heart, and parasite clusters in other organs including the adipose tissue. Unexpectedly, we found evidence for asynchronous amastigote replication within individual host cells. In chronically infected animals, where parasites are mainly restricted to the GI tract, there were no detectable "nests", with infections restricted to single or low numbers of amastigotes per cell. Nests could only be observed in gut tissue once chronically infected animals had been immunosuppressed. For the first time therefore, this approach has allowed us to investigate chronic *T. cruzi* infections at a cellular level. Data will be presented on the host cells within the GI tract during chronic infections and on the replication status of parasites with these reservoir sites.

2D Study of T. cruzi intracellular organization reveals a new host-parasite interaction mediated by the amastigote flagellum.

Lentini, Gaelle (Harvard School of Public Health, USA); Burleigh, Barbara (Harvard School of Public Health, USA)

Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston MA 02115

The obligate intracellular stage of the Chagas disease parasite, *Trypanosoma cruzi*, replicates in the cytoplasm of its mammalian host cell. Previous high-throughput transcriptomic and RNAi screens have highlighted aspects of host metabolism as the lifeline that sustains intracellular *T. cruzi* growth. Despite these advances, characterization of host-parasite interactions at the molecular and cellular level is still pending. We have recently observed that cytosolically-localized *T. cruzi* amastigotes adopt a non-random organization suggesting that the parasite may tether to host cell structures or organelles for functional purposes. Therefore, we studied the correlation between the spatial organization of *T. cruzi* amastigotes and host cytoskeleton and organelles using a variety of imaging methods. This study reveals a close physical proximity between intracellular *T. cruzi* amastigotes and host mitochondria, mediated by the short parasite flagellum. Parasite flagella are often see been 'wrapped' by host mitochondria by 3D super-resolution microscopy and membrane-membrane contacts between both structures have been observed by transmission electron microscopy. This host mitochondria-parasite interaction occurs for several strains of *T. cruzi* as well as in different cell types including human cardiomyocytes. This observation supports the emerging idea that the flagella of Kinetoplastida are sensory organelles that perform specific functions during the different trypanosomatid life stages. In the specific case of *T. cruzi*, our preliminary observation provides the first indication that the short flagellar 'remnant' of intracellular amastigotes may play an important role in metabolic sensing and coupling to the mammalian host cell. Biochemical studies using the recently developed APEX2 proximity-labelling are ongoing to identify the molecular components that mediate this new host-parasite interaction.

2E Adipose tissue is a reservoir of Trypanosoma brucei parasites throughout infection

Trindade, Sandra (Instituto de Medicina Molecular, Lisboa, PRT)

In the mammalian host, *Trypanosoma brucei* occupies the blood and extra-vascular spaces. Using a mouse model, we have previously identified adipose tissue (fat) as a major reservoir harboring phenotypically distinct parasites. The biology of these parasites and their importance for disease remain poorly understood. In this study, our goals were to determine the dynamics of parasite population during the infection and to further characterize the differences between parasites in fat and blood. We started by quantifying the parasite load in blood and gonadal adipose tissue at multiple time points of the infection. Whereas parasitemia underwent typical fluctuations, the occupation of fat remained high throughout the infection varying in total between 10^6-10^7 parasites. By flow cytometry we observed that, whereas fat is populated by slender and stumpy forms, in 8 out of 10 tested days, there was a larger proportion of slender forms in the fat than in the blood (30% and 11 %, respectively), suggesting that adipose tissue may act as a reservoir of slender forms. To compare the differences between slender forms parasites in the fat and blood, we performed a proteomic analysis of monomorphic parasites isolated from these two tissues on day 5 post-infection. We observed that around 15% of genes are differentially expressed, many of which with metabolic roles. These results are consistent with our transcriptomic studies previously published. These studies have confirmed at the protein level that slender parasites in fat and blood are phenotypically different, which may have important implications for drug treatment and understanding of the disease.

2F APOLIPOPROTIEN L-1 in Trypanosome Lytic Factors is highly polymorphic in primates, which governs broad resistance

Verdi, Joey (Hunter College and the Graduate Center at the City University of New York, New York, NY, USA); Thomson, Russell (Hunter College CUNY, USA); Raper, Jayne (Hunter College and the Graduate Center at the City University of New York, USA)

Some primates maintain resistance to infection by *Trypanosoma brucei* via the protective effects of the primate-specific protein apolipoprotein L-1 (APOL1). APOL1 circulates in plasma on high-density lipoprotein complexes called trypanosome lytic factors that are endocytosed by the parasites. Once inside the parasite, the APOL1 forms cation selective pores in membranes leading to osmotic lysis. The human infective *T. b. rhodesiense* and *T. b. gambiense* have independently evolved APOL1 resistance mechanisms. Baboon APOL1 kills *T. b. rhodesiense* by evading binding to its resistance protein (SRA), while the interaction of baboon APOL1 and *T. b. gambiense* remains poorly understood. Whole genome sequencing has recently revealed that the APOL1 gene is highly polymorphic in the human population, with some African-specific variants of APOL1 providing increased resistance to trypanosomes. By analyzing baboon genome sequencing data, from 39 individual baboons we have identified putative APOL1 sequences in five different baboon species, with a high degree of variation within and across species. A variant specific to the *Papio hamadryas* and *Papio papio* species, which both resist infection to *T. b. gambiense* in vivo, exchanges a glutamate residue for a glutamine residue at position 150, relative to the reference sequence Panu2.0. We show that the glutamine at position 150 is required for full protection against infection by trypanosomes in a transiently transgenic mouse.

2G Role of host triglycerides in Trypanosoma cruzi amastigote lipid metabolism

Martin, Jessica L. (Harvard T.H. Chan School of Public Health, Boston, MA, USA); Gazos-Lopes, Felipe (Harvard T.H. Chan School of Public Health, USA); Burleigh, Barbara (Harvard T.H. Chan School of Public Health, USA)

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is the most prevalent parasitic disease in the Americas. However, little is known about the parasite's metabolism, specifically in the amastigote stage, where the parasite resides primarily in the host cell cytosol. Previous studies by our laboratory in which the host cell metabolism was perturbed via RNA interference, illuminated many host pathways supportive of intracellular parasite growth, in particular, lipid metabolism. Indeed, decades of research show a tight association of *T. cruzi* and lipid-rich adipose tissue; however, the molecular causes for this connection remained elusive until recently. Lipidomics studies in our laboratory show that the parasite triacylglycerol (TG) fatty acid (FA) composition mirrors that of its host cell, a feature that was not observed in other major lipid classes, leading to the hypothesis that *T. cruzi* amastigotes scavenge FAs and that the primary source of these FAs is host TGs.

To test these hypotheses, we monitored the incorporation of exogenous ^{14}C-palmitate into different lipid classes of intracellular amastigotes grown in either wild-type or TG biosynthesis-deficient fibroblasts. These studies revealed significantly decreased amastigote incorporation of ^{14}C-palmitate-derived FAs when grown in the TG-compromised host as compared to wild-type. Additionally, chemical inhibition of host adipose triglyceride lipase (ATGL) during infection hindered incorporation of exogenous ^{14}C-palmitate into intracellular amastigotes, suggesting a role for TG hydrolysis prior to fatty acid scavenging by intracellular parasites. Thus, our studies provide preliminary biochemical evidence to support our lipidomics-generated hypotheses that intracellular *T. cruzi* amastigotes scavenge host TG-derived fatty acids. Further studies are ongoing to delineate the precise mechanisms by which the parasite intercepts host TGs.

SESSION III: Gene Expression/

Translation Control
Pegine Walrad, Chair.
Sunday 2:00 pm

04/23/17

3A Variant Surface Glycoprotein gene expression and monoallelic exclusion: how is this controlled?

Rudenko, Gloria (Imperial College London)

Bloodstream form *Trypanosoma brucei* faces relentless immune attack as an extracellular parasite. Key for its survival is a protective and antigenically variable Variant Surface Glycoprotein (VSG) coat. VSG comprises about 10% total *T. brucei* protein, yet is encoded by a single *VSG* gene. How is the trypanosome able to express so much protein from a single gene? An individual trypanosome has a vast wardrobe of thousands of different *VSG*s, of which only one is expressed at a time. The active *VSG* is transcribed from one of about 15 different *VSG* expression site transcription units located at chromosome ends. A highly stringent mechanism of mono-allelic exclusion operates, ensuring that only one *VSG* expression site is active at a time. How does *T. brucei* control this?

Despite the complexity of antigenic variation in *T. brucei,* considerable recent progress has been made in understanding it. Chromatin remodeling has been shown to be key for *VSG* expression site control, and a long list of relevant proteins have been identified. Many of these are factors affecting the epigenetic state of the *VSGs* or their expression sites. Whole genome methods are facilitating the phenotyping of *T. brucei* strains, and allowing the identification of new *VSG* expression site markers. However despite these new insights, some of the most central questions still remain open. The answers that we obtain from this highly divergent eukaryote are guaranteed to be unexpected.

3B Genome-wide chromosome conformation capture (Hi-C) reveals strong compartmentalization of the Trypanosoma brucei genome

Müller, Laura SM (Research Center for Infectious Diseases, University of Würzburg, Germany, USA); Cosentino, Raúl O (Research Center for Infectious Diseases, University of Würzburg, Germany, USA); Förstner, Konrad U (Core Unit Systems Medicine, University of Würzburg, Germany, USA); Lajoie, Bryan R (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA, USA); Kaplan, Noam (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA, USA); Belton, Jon-Matthew (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA, USA); Janzen, Christian J (Department of Cell & Developmental Biology, Biocenter, University of Würzburg, Germany, USA); Steinbiss, Sascha (Parasite Genomics, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK, USA); Otto, Thomas D (Parasite Genomics, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK, USA); Berriman, Matthew (Parasite Genomics, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK, USA); Sebra, Robert P (Icahn Institute and Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, USA, USA); Dekker, Job (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA, USA); Siegel, T Nicolai (Research Center for Infectious Diseases, University of Würzburg, Germany, USA)

Findings in several organisms indicate that regulation of gene expression and the frequency of translocations are intimately linked to the spatial organization of their genomes. In developing B-cells, for example, repositioning of immunoglobulin alleles away from the nuclear lamina is a prerequisite for recombination and monoallelic expression of a single IgH locus and the frequency of translocations between chromosomes in *cis* and *trans* correlate with their relative spatial distances.

In the case of *Trypanosoma brucei* there has been a long-standing interest in deciphering the nuclear organization to better understand the parasite's ability to ensure mutually exclusive expression of variant surface glycoproteins (VSGs).

Thus, to probe the three-dimensional genome structure of *T. brucei*, we performed Hi-C analyses and generated several genome-wide maps displaying the DNA-DNA interactions in bloodstream-form trypanosome nuclei. Hi-C permits assessment of the nuclear architecture by proximity-based ligation of DNA combined with massively parallel sequencing.

Our data reveal the existence of separate chromosome territories, where individual chromosomes occupy a distinct nuclear space. Furthermore, we find the individual chromosomes to be further subcompartmentalized: at first instance, the linear genome is separated into actively transcribed chromosomal cores and transcriptionally silent subtelomeric domains, which mostly contain the repertoire of repressed *VSG* genes. This transcriptional partitioning is also reflected by a differential arrangement of these regions in the 3D nuclear space.

Our findings provide important insights into the 3D DNA architecture in trypanosomes and suggest how nuclear organization may shape gene expression in *T. brucei*.

3C Circadian rhythm controls metabolism in Trypanosoma brucei

Rijo-Ferreira, Filipa (UT Southwestern Medical Center, USA); Pinto-Neves, Daniel (iMM-Lisboa, USA); Barbosa-Morais, Nuno (iMM-Lisboa, USA); Takahashi, Joseph (UT Southwestern Medical Center, USA); Figueiredo, Luisa (iMM-Lisboa, USA)

The Earth's rotation forced life to evolve under day and night environmental cycles. In order to anticipate such daily cycles, prokaryote and eukaryote free-living organisms evolved intrinsic clocks that regulate physiological and behavioral processes. Daily rhythms have been observed in organisms living within hosts, such as parasites. However, whether parasites have intrinsic molecular clocks or whether they simply respond to host rhythmic physiological cues remains a mystery. Here we show that *Trypanosoma brucei*, the causative agent of human sleeping sickness, has an intrinsic circadian clock that regulates its metabolism in two different stages of the life cycle. We found that *in vitro* approximately 10% of genes in *T. brucei* are expressed with a 24h circadian rhythm. The maximum expression of these genes occurs at two different phases of the day. Circadian genes are enriched in cellular metabolic pathways, and coincide with two daily peaks of intracellular ATP concentration. Moreover, circadian changes in the parasite population lead to differences in suramin sensitivity, a drug commonly used to treat this infection. These results demonstrate that *T. brucei* parasites have an intrinsic circadian clock that allows them to keep time independently from their host and that this clock regulates parasite biology throughout the day.

3D Patterns of chromosome copy number change during growth of Leishmania reveal a link between DNA replication and mosaic aneuploidy

Campbell, Samantha (Wellcome Trust Centre for Molecular Parasitology/University of Glasgow, Glasgow, GBR); Duncan, Samuel (University of Dundee, USA); Krasilnikova, Marija (University of Glasgow, USA); Mottram, Jeremy (University of York, USA); McCulloch, Richard (Wellcome Centre for Molecular Parasitology / University of Glasgow, USA); Dickens, Nicholas (FAU Harbor Branch Oceanographic Institute, USA)

Recent studies using marker frequency analysis by genome sequencing (MFA-seq) in *Leishmania* and *Trypanosoma brucei* have revealed differences in the organization of replication initiation sites, termed origins, despite pronounced synteny between the kinetoplastid genomes. Although multiple origins could be mapped by MFA-seq in each *T. brucei* chromosome, only a single major origin was seen in each *Leishmania* chromosome. Genome plasticity, including mosaic aneuploidy, are striking characteristics of the *Leishmania* genome, perhaps allowing the parasite population to adapt to changing environments. However, how aneuploidy arises is poorly understood, including if and how it is connected to genome replication. We have used whole genome sequencing to assess changes that occur at the sequence and structural level as *Leishmania mexicana* promastigote cells are grown in serial passage in culture. We observe that changes in chromosome copy number (ploidy) accumulate during such growth. Strikingly, we find a clear association between ploidy change and chromosome length: chromosomes <1 Mb show increases in ploidy dependent on size, whereas chromosomes >1 Mb progressively decrease in ploidy. We suggest these data are consistent with use of a single constitutive origin in each *L. mexicana* chromosome, reflecting the limits of effective replication from one locus. Furthermore, the data provides a mechanistic explanation for the source of aneuploidy, at least in promastigote cells. We will further describe analysis of gene copy number variation, structural variation and SNP patterns in these conditions. The results of these analyses may be significant in understanding drug resistance mechanisms in *Leishmania*.

3E Elucidating the molecular mechanism for cell cycle-dependent protein translation initiation in trypanosomes

AN, TAI (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, USA); LI, ZIYIN (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA)

Protein translation in eukaryotes is cell cycle dependent. During G1/S phases, translation is robust and is controlled by the cap-dependent translation initiation complex composed of the cap-binding protein eIF4E and the polyadenylate-binding protein PABP. Upon entry into mitosis, however, translation is reduced to basal level and is mediated by the cap-independent translation initiation machinery. This interesting phenomenon has been known for decades, but the underlying mechanism remains largely elusive, and there is no report linking the cell cycle control machinery to the translation initiation machinery. Through chemical genetic approach, we recently identified eIF4E4 and PABP1 as the substrates of the cyclin-dependent kinase CRK1, a crucial G1/S cell cycle regulator in *T. brucei*. Biochemical approaches confirmed that eIF4E4 and PABP1 are *in vivo* substrates of CRK1 and identified seven phosphosites in eIF4E4 and three phosphosites in PABP1. Depletion of eIF4E4 and PABP1 by conditional gene knockout caused significant accumulation of G1 cells, suggesting their essential roles in promoting the G1/S cell cycle transition. Conversely, depletion of CRK1 blocked global protein translation, indicating the requirement of CRK1 for translation initiation. Further, expression of phosphodeficient eIF4E4 and PABP1 mutants inhibited the G1/S transition and impaired protein translation initiation, whereas expression of phosphomimic eIF4E4 and PABP1 mutants restored G1/S cell cycle progression and protein translation, confirming that CRK1-mediated phosphorylation of eIF4E4 and PABP1 is required for the G1/S cell cycle transition and protein translation initiation. Altogether, these findings present, for the first time, the evidence for the intimate relationship between the translation initiation machinery and the cell cycle control machinery, and elucidate the molecular mechanism for the G1/S cell cycle-coupled translation initiation in an early divergent eukaryote.

3F The trypanosome mRNA decapping enzyme is an ApaH-like phosphatase

Kramer, Susanne (Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg, Wuerzburg, DEU); Goos, Carina (Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg, USA)

5'-3' decay is the major mRNA decay pathway in many eukaryotes, including trypanosomes. After deadenylation, mRNAs are decapped by the nudix hydrolase DCP2 of the decapping complex and finally degraded by a 5'-3' exoribnuclease. While deadenylation and 5'-3' exoribonucleic degradation are conserved, Kinetoplastids lack homologues to all subunits of the decapping complex.

Here, we show that trypanosomes use an ApaH-like phosphatase (ALPH1) as their major mRNA decapping enzyme. RNAi depletion of ALPH1 is lethal and causes a massive increase in total mRNAs that are deadenylated but have not yet started 5'-3' degradation. As expected for a decapping enzyme, ALPH1 is present in P-bodies and stress granules. In addition, ALPH1 co-localises exclusively with the 5'-3' exoribonuclease XRNA to a granule at the posterior pole, which is slightly anterior of the microtubules plus end marker XMAP215. While the C-terminus of ALPH1 is needed for the localisation to any RNA granule type, the localisation to the posterior pole is specifically mediated by a sequence motif in the N-terminus. Notably, mRNA decay intermediates do not accumulate at the posterior pole, ruling out that the granule functions in mRNA decay. We propose that, instead, the posterior pole granule may temporally store ALPH1 and XRNA in inactive forms, to dynamically adapt mRNA decay activity to the needs of the cell. The finding that the localisation of ALPH1 to the posterior pole is highly dynamic supports this model: any increase in non-polysomal mRNAs increases the fraction of ALPH1 at the posterior pole, and vice versa, any decrease in non-polysomal mRNAs causes dissociation of ALPH1 from the posterior pole.

This is the first reported function of an ApaH-like phosphatase, a bacterial-derived class of enzymes that are widespread throughout all branches of the eukaryotic kingdom, with the marked exception of vertebrates, land plants and insects. The usage of an ApaH-like phosphatase instead of a nudix domain protein as mRNA decapping enzyme is an example of convergent evolution.

3G Leish4E-IP2 - a novel LeishIF4E-interacting protein involved in stage differentiation

Tupperwar, Nitin (Ben-Gurion University of the Negev, USA); Meleppattu, Shimi (Harvard Medical School, USA); Gilad, Ayelet (Ben-Gurion University of the Negev, USA); Leger-Abraham, Melissa (Harvard Medical School, USA); Wagner, Gerhard (Harvard Medical School, USA); Shapira, Michal (Ben-Gurion University of the Negev, Beer Sheva, ISR)

Translation of most cellular mRNAs in eukaryotes proceeds through a cap-dependent pathway, in which the eIF4F complex assembles on the 5' cap structure and recruits the 40S ribosomal subunit, along with other initiation factors. However, upon exposure to stress conditions, cap-dependent translation is mostly inhibited and translation of a discrete set of genes proceeds via cap-independent mechanisms, which have not yet been well resolved. The trypanosomatid genome encodes six cap-binding proteins (LeishIF4E-1 to -6), which change their expression level and activity during the life cycle. Thus, the ability to modulate the formation of different cap-binding complexes is fundamental for driving stage-specific translation programs. In higher eukaryotes, cap-dependent translation is repressed by the competitive binding of the conserved 4E-Binding protein (4E-BP), which prevents the formation of the translation initiation complex, but is absent in trypanosomatids. However, a set of non-conserved unique and novel 4E-interacting proteins was identified in *Leishmania*, and shown to modulate the formation of different cap-binding complexes. This includes 4E-IP1, which interacts mainly with LeishIF4E-1, and a novel non-conserved 4E-IP2, that interacts with LeishIF4Es. Leish4E-IP2 changes its expression and activity along the growth curve of promastigotes, and Leish4E-IP2 over expression enhances differentiation into axenic amastigotes. Since the different LeishIF4Es have different assigned roles in promastigotes and amastigotes, their interacting proteins (4E-IPs) are expected to play a key role during promastigote growth and during stage differentiation.

3H A novel 'VSG stress response' pathway leads to upregulation of a subset of ER and Golgi proteins during a global translation arrest induced by a VSG synthesis block

Ooi, Cher-Pheng (Imperial College, London, GBR); Wand, Nadina (Imperial College, USA); D'Archivio, Simon (Nottingham University, USA); Gadelha, Catarina (Nottingham University, USA); Wickstead, Bill (Nottingham University, USA); Rudenko, Gloria (Imperial College, USA)

Variant Surface Glycoprotein (VSG) is the most highly expressed protein in bloodstream form *Trypanosoma brucei*, comprising ~10 % total protein. We inhibited VSG synthesis by blocking translation of *VSG* transcript using synthetic morpholinos. This leads to a uniquely precise pre-cytokinesis cell cycle arrest, with cells stalled at 2K2N without re-initiation of S-phase. This argues that VSG synthesis is monitored during the cell cycle, and that blocking it triggers a unique cell cycle checkpoint. The stalled cells undergo a global translation arrest, with protein synthesis reduced to ~60% normal levels after 8 hours, and to 1 – 4 % normal levels after 24 hours induction of *VSG* RNAi. Interestingly, a small subset of proteins are upregulated in the face of this global translation arrest. RNAseq analysis and epitope tagging have allowed us to identify ER and Golgi related genes which are upregulated at both the RNA and protein level after the induction of a VSG synthesis block. These include an ER oxidoreductin which is 3.7x upregulated, and an ER stress chaperone which is 2.5x upregulated at the protein level after 8 hours *VSG* RNAi. In addition, levels of the endoplasmic reticulum (ER) chaperone protein BiP (Binding Immunoglobulin Protein) increase to 1.5x uninduced cells after 8 hours, and 3.4x uninduced cells after 24 h induction of a VSG synthesis block. During the unfolded protein response (UPR) stress response pathway, selected proteins escape a global arrest in protein synthesis. We propose that a unique "VSG stress response" pathway exists, with some superficial similarity to the UPR. We are currently dissecting the molecular mechanisms controlling this pathway.

SESSION IV: Biochemistry/ Drug Discovery
Dan Zilberstein, Chair.
Monday 8:45 am
04/24/17

4A A mitochondrial fucosyltransferase is essential for the viability of Leishmania major

Guo, Hongjie (Washington University School of Medicine, St. Louis, USA); Damerow, Sebastian (University of Dundee, USA); Penha, Luciana (Washington University School of Medicine, St. Louis, USA); Zegzouti, Hicham (Promega Corp., USA); Ferguson, Michael A.J. (University of Dundee, USA); Beverley, Stephen M. (Washington University School of Medicine, St. Louis, USA)

The *L. major* genes *FKP40* and *AFKP80* encode bifunctional proteins with kinase/pyrophosphorylase activities. Both salvage L-fucose to GDP-L-Fuc, but only AFKP80 yielded GDP-D-Ara*p* from D-Arabinopyranose. Correspondingly, Δ*afkp80-* mutants lack D-Ara*p* while Δ*fkp40-* mutants resembled WT. Unexpectedly, we were unable to produce a Δ*fkp40-*/Δ*afkp80-* double mutant. These data raised the possibility that GDP-Fuc (or a related metabolite) is essential.

The *Leishmania* genome predicts at least 5 fucosyltransferases, of which LmjF01.0100 is the only one conserved amongst trypanosomatids, showing key motifs characteristic of α-1,2 fucosyltransferases. Enzymatic assays of *L. major* overexpressing C-terminally tagged Lm01 showed transfer of Fuc from GDP-Fuc to a Galβ1,3GlcNAc-β-O-Methyl acceptor. To test essentiality, we used a quantitative 'plasmid segregation' assay, expressing *Lm01* from the multicopy episomal pXNG vector, which additionally expresses GFP, in a chromosomal null Δ*lm01*$^-$ background. Single cell sorting for GFP-null lines rarely yielded viable cells, indicating that *Lm01* is essential.

Unlike most eukaryotic glycosyltransferases, Lm01 was predicted to localize to the mitochondrion, which was confirmed experimentally by expression of a C-terminally tagged protein (Lm01-HA). To probe the importance of mitochondrial localization, we used 'plasmid shuffling', asking whether pXNG-*Lm01* could be lost from Δ*lm01*$^-$ in the presence of mutated test *Lm01* genes bearing a C-terminal HA tag. A variety of modifications affecting mitochondrial localization could not rescue the Δ*lm01*$^-$ mutant; each of these proteins was expressed at high levels but were now found in the cytosol at high levels and showing WT recombinant enzyme activity. Mutation of the predicted FucT catalytic motif resulted in loss of function and enzymatic activity, while maintaining mitochondrial localization. Together these results suggest that both mitochondrial localization and FucT activity are essential for Lm01 functionality. Correspondingly, the rare Δ*lm01*$^-$ survivor was quite sick and showed a variety of severe mitochondrial defects. Thus, the Lm01 FUT1 joins the eukaryotic O-GlcNAc transferases as one of the first glycosyltransferases shown to act within the mitochondrion. Current work is now oriented towards identifying the *Leishmania* fucosylated targets therein.

4B Assembly and function of BILBO1, a multidomain cytoskeletal protein at the flagellar pocket collar of Trypanosoma brucei

Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria, Vienna, WN, AUT)

Trypanosoma brucei is a protozoan parasite causing sleeping sickness in Africa. At the base of its single flagellum is a bulb-like structure called the flagellar pocket (FP), which is responsible for all endo-/exocytosis of the cell and thus essential for the survival of the parasite. At the neck of the FP is an electron-dense structure called the flagellar pocket collar (FPC), which is essential for FP biogenesis and thus parasite survival. BILBO1 was the first reported protein component of the FPC. We have characterized the structure and assembly of this multidomain cytoskeletal protein. Briefly, our initial bioinformatic analysis suggests that the 67-kDa BILBO1 consists of four structural domains, including a globular N-terminal domain, two central EF-hand motifs followed by a long coiled-coil domain, and a C-terminal leucine zipper. We found that recombinant BILBO1 forms enormously large oligomers *in vitro*, which makes it intractable by any single conventional structural study method. Therefore, we carried out structural dissection of *T. brucei* BILBO1 using integrative structural biology approaches including NMR, crystallography, EM, and various biophysical methods. The high-resolution structure of its N-terminal domain (by both NMR and X-ray) reveals a variant ubiquitin-like fold with a unique conserved surface patch; mutagenesis of this patch causes cell death *in vivo*. We further found that the EF-hand motifs change their conformation upon calcium binding, whereas the coiled-coil domain forms an anti-parallel homodimer. Employing a series of truncation analyses and EM experiments, we revealed that intermolecular interactions between adjacent leucine zippers allow BILBO1 to form extended filaments. These filaments were additionally shown to condense into fibrous bundles through lateral interactions. Based on all these experimental data, we propose a mechanism for BILBO1 assembly at the flagellar pocket collar, which enables it to assume the role as a scaffold of the FPC.

4C Aquaglyceroporin-null trypanosomes display glycerol transport defects and respiratory-inhibitor hypersensitivity

Jeacock, Laura (University of Dundee, USA); Baker, Nicola (University of York, USA); Wiedemar, Natalie (Swiss Tropical and Public Health Institute & University of Basel, USA); Mäser, Pascal (Swiss Tropical and Public Health Institute & University of Basel, USA); Horn, David (University of Dundee, USA)

Aquaglyceroporins (AQPs) transport water and glycerol and play important roles in drug-uptake in pathogenic trypanosomatids. For example, *AQP2* in the human-infectious African trypanosome, *Trypanosoma brucei gambiense*, is responsible for melarsoprol and pentamidine-uptake, and melarsoprol treatment-failure has been found to be due to *AQP2*-defects in these parasites. To further probe the roles of these transporters, we assembled a *T. b. brucei* strain lacking all three *AQP*-genes. Triple-null *aqp1-2-3 T. b. brucei* displayed only a very moderate growth defect *in vitro*, established infections in mice and recovered effectively from hypotonic-shock. The *aqp1-2-3* trypanosomes did, however, display glycerol uptake and efflux defects; they failed to utilise glycerol as a carbon-source and displayed increased sensitivity to salicylhydroxamic acid (SHAM) and propyl gallate; both inhibitors of trypanosome alternative oxidase (TAO) that increase intracellular glycerol. Notably, disruption of *AQP2* alone generated cells with glycerol transport defects. Consistent with these findings, AQP2-defective, melarsoprol-resistant clinical isolates were also TAO-inhibitor hypersensitive. Thus, African trypanosome AQPs are not required for viability or osmoregulation but do make important contributions to drug-uptake and glycerol-flux. This improved understanding of AQP-function, and AQP2-mediated drug-uptake and glycerol-efflux in particular, could be exploited. For example, therapies combining TAO-inhibitors with melarsoprol or pentamidine could be more effective than previously anticipated and could mitigate the further emergence and spread of melarsoprol-resistance.

4D A chemical tool to study signaling from the unconventional PKA-like kinase of Trypanosoma brucei

Bachmaier, Sabine (Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany, Martinsried, DEU); Kramer, Susanne# (Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany, USA); Githure, George (Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany, USA); Volpato, Yuri (Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany, USA); Pepperl, Julia (Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany, USA); Dupuy, Jean-William (Centre de Génomique Fonctionnelle Bordeaux, Université Bordeaux, France, USA); Bonneu, Marc (Centre de Génomique Fonctionnelle Bordeaux, Université Bordeaux, France, USA); Genieser, Hans-Gottfried (BIOLOG Life Science Institute, Bremen, Germany, USA); Schwede, Frank (BIOLOG Life Science Institute, Bremen, Germany, USA); Boshart, Michael (Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany, USA)*

The protein kinase A (PKA) signaling pathway is highly conserved throughout the eukaryotic kingdom. Activation by cyclic AMP and regulation of a plethora of cellular processes such as metabolism, differentiation or cell cycle progression, are hallmarks of PKA in unicellular as well as multicellular organisms. We have previously shown that the PKA signaling pathway has been retooled in the evolutionary early branching eukaryotic parasite *Trypanosoma brucei*. The parasite PKA is not activated by the second messenger cAMP, while otherwise exhibiting characteristic PKA features. Here we report identification of several 7-deazapurine derivatives as potent activators of *T. brucei* PKA by a small-scale chemical screen. Further optimization of the best hit led to improvement in specificity and lower nM affinity and activation potency. These potent compounds are novel tools that we used for quantitative proteome analysis to identify downstream targets of PKA signaling in *T. brucei*. Furthermore, the essentiality of the kinase in *T. brucei* and the highly similar orthologues of PKA in the related kinetoplastids *Trypanosoma cruzi* and *Leishmania donovani* suggest that the lead compound may be further developed to target this PKA-like kinase with unique kinetoplastid-specific properties for treatment of kinetoplastid-caused diseases.

* corresponding author: boshart@lmu.de

present address: Department of Cell & Developmental Biology, Biocenter of the University of Würzburg, Würzburg, Germany

4E A potential mode of action of the anti-trypanosomal benzoxaborole AN7973

Begolo, Daniela (ZMBH, Heidelberg University, USA); Barrett, Michael P. (WCMP, University of Glasgow, USA); Clayton, Christine (ZMBH, Heidelberg University, USA)

In the past few years several million compounds have been tested for selective toxicity towards kinetoplastids, and thousands have shown activity. To develop such compounds, it is useful to know the mode of action. Characterization of cells that become resistant after RNAi has proven extremely useful in identifying mechanisms of uptake or intracellular activation, but not, so far, in identifying molecular targets. The benzoxaborole compound AN7973 was considered for development for cattle trypanosomiasis, and a quite similar compound, AN5568, is in phase II trials for sleeping sickness. We investigated the mode of action of AN7973 using multiple methods, including screening of an over-expression library, genome sequencing of resistant mutants, metabolome analysis, morphological observations and measurements of macromolecular biosynthesis. The results, which will be presented, reveal a novel mode of action which may explain the potency of AN7973 against trypanosomes.

4F A FRET Cytometry High Throughput Screen to Identify Specific Inhibitors of Glucose Uptake and Distribution in Trypanosoma brucei

Voyton, Charles (Clemson University, Provo, UT, USA); Ackroyd, P. Christine (Brigham Young University, USA); Morris, Meredith (Clemson University, USA); Morris, James (Clemson University, USA); Christensen, Kenneth (Brigham Young University, USA)

Glucose metabolism via glycolysis in glycosomes is the sole source of ATP for the bloodstream form of the African trypanosome, *Trypanosoma brucei*. The requirement of glucose for survival of this mammalian infectious stage has long made its uptake and metabolism promising targets for anti-trypanosome activity. Current drug screening technologies largely rely on whole cell viability assays, or enzyme inhibition assays, both of which lack the specificity needed to identify inhibitory compounds that specifically inhibit glucose uptake. Here we describe a method that employs an endogenously expressed fluorescent biosensor in cytosol (FLII12PGlu-600u) and glycosomes (PTS-Flip600) of insect form (PCF) and mammalian infectious form (BSF) *T. brucei* that allows monitoring of glucose levels in living parasites due to changes in external environment or addition of a small molecule. Using sensor readout as a metric for intracellular glucose concentration, we can score the efficacy of candidate compounds inhibition of glucose uptake into cytosol and glycosomes in real time. Using this methodology in tandem with flow cytometry, we have developed a robust and specific high throughput screen that can identify glucose uptake inhibitors at a rate of 250 compounds per hour with a Z-prime value greater than 0.9 for PCF parasites. Using Pathogen Box, a curated library specific for neglected diseases, we were able to validate our screen by identifying compounds that inhibit transport of glucose into the cytosol and glycosomes specifically with IC50 values of 5 μM and 1 μM respectively. The identified compounds were validated against BSF parasites with IC50 values of 10 μM. Expanding on this pilot screen, we plan to screen 50,000 compounds to identify novel high affinity glucose uptake inhibitors.

4G Selective inhibition of RNA polymerase I transcription as a potential approach to treat African trypanosomiasis

Kerry, Louise Elizabeth (Imperial College London, USA); Pegg, Elaine E (Imperial College London, USA); Cameron, Donald P (Peter MacCallum Cancer Centre, Australia, USA); Budzak, James (Imperial College London, USA); Poortinga, Gretchen (Peter MacCallum Cancer Centre, Australia, USA); Hannan, Kate (Peter MacCallum Cancer Centre, Australia, USA); Hannan, Ross D (Peter MacCallum Cancer Centre, Australia, USA); Rudenko, Gloria (Imperial College London, USA)

Trypanosoma brucei relies on an essential Variant Surface Glycoprotein (VSG) coat for survival in the mammalian bloodstream. High *VSG* expression within an expression site body (ESB) is mediated by RNA polymerase I (Pol I). The Pol I transcription inhibitors quarfloxin, CX-5461, and BMH-21 are currently under investigation for cancer chemotherapy. As *T. brucei* is reliant on Pol I for VSG transcription, we investigated these inhibitors for selective anti-trypanosomal activity. In *T. brucei* all three Pol I inhibitors have IC50 concentrations for cell proliferation in the nanomolar range: quarfloxin (155 nM), CX-5461 (279 nM) or BMH-21 (134 nM). *T. brucei* was therefore 29-fold more sensitive to quarfloxin, 25-fold more sensitive to CX-5461 and 3.4-fold more sensitive to BMH-21 than the human MCF10A cell line. Cell death in *T. brucei* was due to rapid and specific inhibition of Pol I transcription, and within 15 minutes treatment rRNA precursor transcript was reduced 97-98% and *VSG* precursor transcript 91-94%. Incubation resulted in disintegration of the ESB as well as the nucleolus subnuclear structure within one hour. Rapid ESB loss following the block in Pol I transcription argues that the ESB is a Pol I transcription nucleated structure, similar to the nucleolus. While Quarfloxin and CX-5461 treatment was irreversible, wash-out of the BMH-21 inhibitor ($\leq 1\,\mu M$) resulted in restored *T. brucei* proliferation. Although this could limit the potential of BMH-21 as a therapeutic agent, it provides a valuable tool for investigating the kinetics of ESB assembly. In addition to providing insight into Pol I transcription and ES control, Pol I transcription inhibitors potentially also provide new approaches to treat trypanosomiasis.

4H Glycosomal hypertrophy and the response to suramin in African trypanosomes

Zoltner, Martin (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom, Dundee, GBR); Vaughan, Sue (Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom, USA); Gadelha, Catarina (Queen's Medical Centre, University of Nottingham, Nottingham, United Kingdom, USA); Leung, Ka-Fai (Department of Pathology, University of Cambridge, Cambridge, United Kingdom, USA); Güther, Maria Lucia S. (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom, USA); Burrell, Alana (Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom, USA); Ali, Liaqat (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom, USA); Ferguson, Michael A. J. (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom, USA); Field, Mark C. (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom, USA)

Suramin, a key trypanosomiasis drug developed over a century ago, remains in the clinic for treatment of early stage disease, while its mode of action is elusive. Recent studies suggest a prominent role of endocytosis for suramin uptake and we investigated the effect of suramin on *Trypanosoma brucei* at the ultrastructural level using transmission and serial-block-phase scanning electron microscopy. After two days of suramin treatment, cells begin to exhibit morphological abnormalities, which included enlargement of the flagellar pocket. However, the most striking alteration was a significant increase in the volume of glycosomes. Surprisingly, suramin did not lead to a corresponding increase of glycosomal proteins, but a decrease in the glycosomal UDP-glucose 4'-isomerase TbGalE and homologs of peroxisome biogenesis factors 2 and 12. The most prominent protein abundance changes were upregulation of the plasma membrane glucose transporter THT1 and a protein cohort involved in differentiation to stumpy form, including PAD isoforms and the receptor-type adenylyl cyclase GRESAG4. Analysis of sugar nucleotide pools revealed reduced levels of UDP-α-D-*N*-acetylglucosamine upon suramin treatment, while the four remaining sugar nucleotides were not significantly perturbed. Our data identify for the first time a specific metabolic process impacted by suramin.

SESSION V: RNA Biology
Sara Zimmer, Chair.
Tuesday 8:45 am
04/25/17

5A A role for retrotransposon hotspot proteins in transcription in Trypanosoma brucei?

Florini, Francesca (IZB, University of Bern, Bern, CHE); Naguleswaran, Arunasalam (IZB, University of Bern, USA); Bringaud, Frédéric (University of Bordeaux, USA); Roditi, Isabel (IZB, University of Bern, USA)

Retrotransposon hotspot proteins constitute 7 sub-families of (predominantly) nuclear proteins in *T. brucei* (RHS1-7). Related proteins are found in *T. congolense, T. evansi* and *T. cruzi*, but not in *Leishmania*. It was previously shown that several sub-families are associated with the chromatin modulator TbRMM1 (1). We have focused on RHS6, which consists of a single gene, and RHS4, which associates with RNA polymerase II (RNAPII; ref.2). Depletion of either RHS4 or RHS6 by RNAi resulted in growth arrest of procyclic forms. Chromatin-immunoprecipitation (ChIP)-Seq experiments showed that both RHS4 and RHS6 delineate polycistronic units transcribed by RNAPII. By contrast, they are depleted at transcription start sites, as well as regions of the genome that are transcribed by RNAPI (procyclin and rRNA loci), and silent regions such as internal clusters of VSG and expression site-associated genes. Actively transcribing RNAPII associates with RHS 1,3,4,5 and 6 family members, whereas RNAPII inhibited by actinomycin D associates only with RHS4 and RHS5. Immunoprecipitation of RHS6 revealed that, in addition to other RHS and several subunits of RNAPII, the protein interacts with components likely to function in RNA processing, transcription elongation and nuclear export. Incorporation of the nucleoside analogue 5-ethynyluridine will allow us to determine if RHS proteins play a direct role in transcription.

1. Naguleswaran et al, *Trypanosoma brucei* RRM1 Is a Nuclear RNA-Binding Protein and Modulator of Chromatin Structure, 2015, mBio, 6(2):1-11
2. Das et al, Biochemical characterization of *Trypanosoma brucei* RNA polymerase II, 2006, Molecular and Biochemical Parasitology, 150(2): 201-210

5B The vault RNA of Trypanosoma brucei is required for the production of trans-spliced mRNA

Kolev, Nikolay G. (Yale School of Public Health, New Haven, CT, USA, USA); Rajan, K. Shanmugha (Bar-Ilan University, Ramat-Gan, Israel, USA); Tycowski, Kazimierz T. (Yale School of Medicine, New Haven, CT, USA, USA); Toh, Justin Y. (Yale School of Public Health, New Haven, CT, USA, USA); Shi, Huafang (Yale School of Public Health, New Haven, CT, USA, USA); Lei, Yuling (Yale School of Public Health, New Haven, CT, USA, USA); Michaeli, Shulamit (Bar-Ilan University, Ramat-Gan, Israel, USA); Tschudi, Christian (Yale School of Public Health, New Haven, CT, USA, USA)

The vault RNP, comprised of the vault RNA (vtRNA) and telomerase-associated protein 1 (TEP1), is found in many eukaryotes, but studies of vtRNAs in mammalian cells have so far not reached a definitive conclusion about its function. VtRNAs are related to Y RNAs, which are complexed with Ro60 protein (Wolin *et al*., RNA Biol. 2013). Ro60 is ring shaped and its central cavity binds the single-stranded ends of misfolded noncoding RNAs (ncRNAs). The Y RNA interacts with the outer ring surface and can influence Ro60's function in ncRNA quality control. In *T. brucei*, TbsRNA10 was first described in a survey of the ncRNA repertoire (Michaeli *et al*., Nucleic Acids Res. 2012). We show that TbsRNA10 is the trypanosome vtRNA and like all vtRNAs, it is transcribed by Pol III and interacts with TEP1. In contrast to other eukaryotes, the abundance of the trypanosome vtRNA is comparable to the levels of spliceosomal snRNAs and it is enriched in a nuclear compartment reminiscent of a Cajal body. It is essential and its levels change during the parasite life cycle. Although vtRNAs exhibit little sequence conservation, we describe a short sequence element conserved in all vtRNAs. This sequence motif resembles a U-rich pre-mRNA element shown in *C. elegans* to be important for *trans*-splicing of nematode polycistronic mRNA precursors. In a permeabilized cell system for bloodstream form *T. brucei* we discovered that decreasing the vtRNA levels leads to impaired *trans*-splicing of newly transcribed pre-mRNA without a similar effect on polyadenylation. Our results suggest a common theme for the function of vtRNAs and Y RNAs, namely involvement in the processing of noncoding and coding RNA precursors.

5C Dual pseudouridylation on rRNA and snRNAs and the role of hyper-pseudouridylation during the life cycle of Trypanosoma brucei

Rajan, K. Shanmugha (Bar-Ilan University Israel, USA); Chikne, Vaibhav (Bar-Ilan university, USA); Doniger, Tirza (Bar-Ilan university, Israel, USA); Cohen Chalamish,, Smadar (Bar-Ilan University, USA); Unger, Ron (Bar-Ilan University, USA); Tschudi, Christian (Yale University, USA); Michaeli, shulamit (Bar-Ilan university, USA)

We recently applied the Psi-seq genome-wide mapping of pseudouridines (Ψ) in procylic and bloodstream parasites on rRNA, and identified 83 H/ACA small nucleolar RNAs (snoRNA), which guide these modifications. We also provided evidence that pseudouridylation of rRNA plays an important role in the parasite transition between the insect midgut and the mammalian bloodstream and observed an elevation of 21 Ψs in bloodstream forms as a result of increased levels of the guiding snoRNAs (Chikne et al., Sci.Rep. 2016). Here, we present the first extensive genome-wide mapping of this modification on small RNAs. We identified the highest number of Ψs (68) on snRNAs, as compared to 27 in mammals and 8 in yeast. The modification was shown to affect the stability of U4/U6 interaction and in the two life stages. The relatively small number of H/ACA snoRNAs compared to at least 136 sites we mapped on rRNA and snRNAs suggests that most of the snoRNAs guide the modification on more than a single target. This extensive dual functionality seems to be unique to trypanosomes, since in H/ACAs are composed of a single hairpin structure as opposed to two in other eukaryotes. Using snoRNAi we provide evidence for the dual functionality of the snoRNAs. We show that these RNAs are located in a special body near the nucleolus, reminiscent of a Cajal-like body. We found that the binding of kinetoplastid- specific protein designated methyltransferase-associated protein (TbMTAP) converts a snoRNA that guide modification on rRNA to an snRNA guider. The hypermodification of Ψs will be discussed with its implication on ribosome and spliceosome function in the two life cycle stages of the parasite.

5D Metabolism and the maxicircle genome: exploring potential relationships using Trypanosoma cruzi strain variability

Kalem, Murat C. (Biomedical Sciences, University of Minnesota Medical School, Duluth, MN, USA, Duluth, MN, USA); Flegontov, Pavel (University of Ostrava, Ostrava, CZE, USA); Gerasimov, Evgeny (Moscow State University, Moscow, RUS, USA); Rasmussen, Bailey (Biomedical Sciences, University of Minnesota Medical School, Duluth, MN, USA, USA); Susa, Emily (Biomedical Sciences, University of Minnesota Medical School, Duluth, MN, USA, USA); Zimmer, Sara L. (Biomedical Sciences, University of Minnesota Medical School, Duluth, MN, USA, USA)

Mitochondrial maxicircle genomes possess many insertions, deletions, and substitutions among *Trypanosoma cruzi* strains. Concomitantly, pioneering studies have noted strain-specific metabolic differences. We hypothesize that differences in *T. cruzi* cellular function arise from variation in maxicircle sequences and their expression. Therefore, we investigated metabolic parameters potentially sensitive to maxicircle gene product abundances in strains with sequenced maxicircles. Axenic culture epimastigotes of all strains depleted glucose at similar rates, but there were differences in medium pH following glucose depletion, suggesting differences in excreted byproducts. To determine whether there were strain-specific differences in the respiratory pathway, oxygen consumption and membrane potential were investigated. Strain Esmeraldo exhibited the greatest medium pH decrease. Interestingly, Seahorse Bioanalyzer experiments revealed that it also had the least basal respiratory capacity, ATP-coupled respiration, and spare respiratory capacity of all strains. The converse was true for strain CL Brener, and Sylvio X-10 had an intermediate phenotype. We then addressed the possibility that these strain-specific metabolic differences may originate in maxicircle gene expression. To establish baseline maxicircle expression patterns we performed RNA-seq and processed reads with the TAligner3 program designed for mapping reads with multiple U-insertions/deletions. Compellingly, the read ratio of maxicircle derived edited mRNAs relative to their pre-edited counterparts was dramatically less in Esmeraldo than in CL Brener where it was highest, with reads from Sylvio X-10 again phenotypically intermediate. Other differences such as upregulated COIII abundance in CL Brener, and upregulated ND8 abundance in Esmeraldo and Sylvio X-10 suggest fine-tuning of individual maxicircle transcript expression. In conclusion, our data are consistent with a model in which degree of maxicircle editing regulates *T. cruzi* epimastigote metabolic potential.

5E Early life of mitochondrial RNAs in Trypanosoma brucei

Sement, Francois (Boston University, Boston, MA, USA); Zhang, Liye (USA); Afasizheva, Inna (USA); Afasizhev, Ruslan (USA)

Historically, the mechanisms of mitochondrial gene expression have been studied in the context of mRNA editing and polyadenylation, while the upstream processes of maxicircle transcription and primary RNA processing remained virtually unexplored. It is commonly held that both kinetoplast DNA strands are transcribed into polycistronic precursors that undergo endonucleolytic cleavage to produce individual pre-mRNAs. This notion is supported by the monophosphorylated state of mature mRNA 5′ ends, but the processing endonuclease is unknown. By combining *in vivo* UV-crosslinking and deep sequencing, we demonstrate that transcription starts at the 5' end of each maxicircle gene. Furthermore, accumulation of mRNA precursors in knockdowns of 3′ processome (MPsome) components indicate that each mRNA is independently transcribed as a 3′ extended precursor, and processed by uridylation-induced 3′-5′ degradation. In addition, we investigated the mechanism by which transcription-defined 5′ terminus is processed from primary triphosphorylated to the mature monophosphorylated state. We show that the mitochondrial edited RNA stability (MERS) complex containing MERS1 NUDIX hydrolase and MERS2 pentatricopeptide-repeat containing RNA binding protein is tightly associated with mRNA 5' ends. Finally, we provide evidence that MERS1's pyrophosphohydrolase activity is essential for parasite viability. In this work, we present a model in which each maxicircle-encoded RNA is transcribed from gene-specific promoter as a 3' extended precursor. This primary transcript is then processed by anti-sense RNA-controlled, MPsome-catalyzed 3′-5′ degradation prior to polyadenylation. We postulate that the transcription-defined 5'end remains bound by the MERS complex acting as "protein cap".

5F A catalytic RNA helicase subcomplex in the trypanosome holo-editosome

Doharey, Pawan (Texas A&M University, USA); Kumar, Vikas (Texas A&M University, USA); Gulati, Shelly (University of Oklahoma Health Sciences Cente, USA); Wohlschlegel, James (University of California, USA); Schnaufer, Achim (University of Edinburgh, USA); Mooers, Blaine (University of Oklahoma Health Sciences Center, USA); Cruz-Reyes, Jorge (Texas A&M UNiversity, USA)

Trypanosomes are flagellated protozoa that split from other eukaryotic lineages over 100 Ma and exhibit extensive RNA editing by uridylate insertion and deletion. In *T. brucei*, hundreds of small non-coding guide RNAs (gRNAs) modify over 1300 sites in the mitochondrial mRNAome. This process takes place in holo-editosomes that include the RNA editing enzyme (RECC) and auxiliary RNA-protein subcomplexes. Yet, the regulatory mechanisms of RNA editing remain obscure.

We recently reported the first catalytic accessory subcomplex, termed the REH2C, which includes mRNA substrates and products, the multi-domain RNA Editing Helicase 2 (REH2) and an intriguing 8-zinc finger protein that we termed REH2-Associated Factor 1 (^{H2}F1). Both proteins are critical for editing *in vivo*. In trypanosomes, ^{H2}F1 stabilizes REH2 and is an adaptor that tethers the helicase into editosomes.

We now reconstituted a catalytically active REH2C using full-length recombinant proteins: REH2 (242 kDa) and ^{H2}F1 (62 kDa). We performed the first biochemical characterization of the REH2C subcomplex, including RNA unwinding, RNA binding, and structural proteomics. We also performed extensive mutagenesis *in vivo* and *in vitro* dissecting features in REH2 and ^{H2}F1 that participate in the assembly of REH2C, its docking into editosomes, and overall editing function. Our RNA-seq studies indicate that the REH2 helicase controls editing fidelity. In summary, we will present comprehensive genetic, biochemical, proteomic and bioinformatic studies of the only known regulatory helicase subcomplex in holo-editosomes.

5G The Leishmania mRNA-bound proteome changes throughout the lifecycle; candidate regulators of parasite differentiation

de Pablos Torro, Luis M. (Centre for Immun. and Infection, Univ. or York, USA); Ferreira, Tiago R. (Centre for Immun. and Infection, Univ. of York, USA); Dowle, Adam (Metabolomics and Proteomics Lab, Biol. Tech Fac., Univ. of York, USA); Walrad, Pegine B. (Centre for Immun. and Infection, Univ. of York, University of York, GBR)

Leishmania spp. parasites adapt to drastic changes in environments during their transmission between insect vectors and human hosts. These adaptions require precise gene regulation to coordinate the timely expression of lifecycle stage-specific proteins essential for survival. Gene regulation in *Leishmania* spp. parasites demonstrates near complete reliance on post-transcriptional genetic control, yet insight into *trans*-regulators which control lifecycle progression is limited. In comparison to Trypanosomes, the molecular regulators behind *Leishmania* differentiation remain elusive.

To isolate key *trans*-regulators implicit in *L.mexicana* parasite differentiation and infectivity, we crosslinked RNA binding proteins (RBPs) to bound mRNAs *in vivo* using an optimised method with reduced stress, then quantitatively identified the mRNA-bound proteome of culture-derived procyclic and metacyclic promastigotes and macrophage- and lesion-derived amastigotes using mass spectroscopy. To verify RBP expression profiles we endogenously tagged genes of differentially-expressed RBPs and performed western blot analyses using constitutive standards. We are currently examining subcellular localisation of endogenously tagged RBPs, genetically characterizing potential regulatory function and isolating associating RNAs for relevance to differentiation and infectivity.

Results reveal a comprehensive list of developmentally-regulated RBPs with distinct expression profiles. Gene Ontology (GO) term analysis revealed a pronounced enrichment of terms associated with gene regulation and RNA binding. Some non-canonical putative RNA interactors verified in other eukaryotic systems were also identified in our screen. Endogenously tagged RBPs confirmed stage-specific protein expression and showed interesting variance to RNA levels. Stage-specific control of transcript stability and translation support a role for identified RBPs in parasite developmental progression and associated virulence. Our investigation into stage-specific RBPs will provide novel insight into *trans*-regulatory mRNP complexes which drive lifecycle progression to human-infective forms.

SESSION VI: Cell Biology II
Ziyin Li, Chair.
Tuesday 2:00 pm

04/25/17

6A Differentiation and quorum sensing in bloodstream form African trypanosomes

Keith R. Matthews, (University of Edinburgh, UK)

In the bloodstream of mammalian hosts, African trypanosomes regulate their proliferation through the generation of stumpy forms, which are also adapted for transmission to tsetse flies. The development to stumpy forms is mediated by a quorum-sensing signal apparently released by the parasites themselves and this results in cell cycle arrest, morphological transformation and the acquisition of competence for differentiation to the procyclic stage. In the presentation I will discuss the evidence for stumpy forms being the transmission stage of the parasite, the molecular components that contribute to their recognition of entry in to the fly and also the dissection of the signalling pathways that mediate the production of stumpy forms. At the end of the presentation I will discuss how the biology and mechanisms of stumpy formation interact with other contributors to the infection dynamic (antigenic variation and compartmentation), and also the presence of co-infecting parasites, to highlight the elegant balance of virulence and transmission in the life cycle of trypanosomes in the bloodstream.

6B Life-stage specific cargo receptors facilitate GPI-surface coat protein transport in Trypanosoma brucei

Kruzel, Emilia K. (University at Buffalo, SUNY, Buffalo, NY, USA); Zimmett, George (USA); Bangs, James (USA)

In African trypanosomes, trafficking of the GPI-anchored Variant Surface Glycoprotein (VSG) to the cell surface is critical to parasite survival and pathogenesis. VSG is synthesized in the ER and then transported to the Golgi apparatus in Coat Protein II (COPII)-coated vesicles. Efficient ER exit of VSG is GPI-dependent and is mediated by specific COPII subunits (TbSec23.2/24.1), suggesting a sorting receptor that simultaneously recognizes the GPI anchor (lumenal-face) and assembling COPII subunits (cytoplasmic face) at ER exit sites (ERES). In other systems, this process is carried out by members of the p24 family of transmembrane proteins, and we hypothesize that trypanosomal p24 orthologues function to select VSG for incorporation into forming COPII vesicles. We have identified 8 putative p24 genes in the *T. brucei* genome: TbERP1-8 (EMP24-Related Protein). Of these, only TbERP1, 2, 3, and 8 are expressed during bloodstream-form growth, and RNAi silencing of each delayed transport of VSG and other GPI-anchored reporters. Immunofluorescence confirmed TbERP co-localization at Sec24.1-positive ERES. TbERP1,2,3,8 are also dependent on one another for stability. Specific RNAi knockdown of any single TbERP affects the steady state levels of the others, suggesting function within a single oligomeric complex that acts as the ER GPI-cargo receptor. Procyclic-form (insect stage) trypanosomes are covered by a surface coat of GPI-anchored Procyclin, which has a distinct GPI-anchor structure. A separate cohort of TbERPs (1,2,4,8) are expressed during this stage, and RNAi silencing of these again confirmed a role in GPI-dependent transport. Collectively, these results suggest that regulated TbERP cohorts, likely in distinct complexes, recognize stage-specific GPI-anchors to facilitate export of GPI-cargo from the ER.

6C A GPR89 family surface protein is an oligopeptide transporter that signals slender to stumpy differentiation in T.brucei

Rojas, Federico (University of EdinburghCentre for Immunity, Infection and Evolution, Institute for Immunology and infection Research, School of Biological Sciences, A, Edinburgh, GBR); Thompson, Joanne (USA); R. Matthews, Keith (USA)

The differentiation from slender forms to stumpy forms in *Trypanosoma brucei* is a density-dependent response that resembles quorum sensing in microbial systems and is crucial for parasite life-cycle development. The stumpy induction factor/s (SIF) and its surface receptor have remained unidentified. We have identified a *T. brucei* protein (TbGPR89) related to eukaryotic GPR89 family members implicated in abscisic acid signalling in plants and Golgi acidification in mammals. TbGPR89 is expressed on the surface of slender forms but is lost as cells differentiate to stumpy forms. Inducible overexpression of TbGPR89 in pleomorphic parasites, but not monomorphs, drives differentiation to stumpy forms *in vitro* and *in vivo*. This response is lost when genes required for SIF-induced stumpy formation are silenced by RNAi, demonstrating signalling through the same pathway. I-TASSER structural homology analysis revealed that the TbGPR89 predicted structure aligns almost perfectly with the structure of proton-coupled oligopeptide transporter (POT) family members. These are present through prokaryotic and eukaryotic organisms, including kinetoplastids, but specifically absent in African trypanosome species. Conditional KO shows that TbGPR89 is essential for parasite growth. Expression of TbGPR89 in bacteria shows specific uptake of the fluorescent Di-peptide ß-Ala–Lys (AMCA), whilst mutation of a key amino acid predicted by homology modelling to be important for oligopeptide transport reduced dipeptide uptake by 50%. Furthermore, inducible expression of a bacterial POT in pleomorphic trypanosomes leads to plasma membrane localisation and drives stumpy formation. Our experiments demonstrate that TbGPR89 is an oligopeptide transporter, essential for parasite viability, that can also uptake an external stimulus leading to stumpy formation.

6D Tubulin poly-glutamylation modulates intraflagellar transport in the Trypanosoma brucei flagellum

Lemos, Moara (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, Paris, France, USA); Jung, Jamin (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, USA); Blisnick, Thierry (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, USA); Fort, Cécile (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, USA); Carsten, Janke (Institut Curie, PSL Research University, CNRS UMR3348, F-91405, Orsay, France, USA); Bastin, Philippe (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, Paris, France, USA)

The *Trypanosoma brucei* flagellum axoneme is composed of microtubule doublets in a 9+2 structure and of a Paraflagellar Rod, a lattice-like structure associated to the axoneme. This organelle is assembled by intraflagellar transport (IFT), a bi-directional movement of protein complexes driven by kinesin (anterograde transport) and dynein (retrograde transport) motors along microtubule doublets. Using Focused Ion Beam Scanning Electron Microscopy, we observed that IFT trains are restricted to doublets 4 and 7 of the *T. brucei* flagellum. What could differentiate these two doublets from the remaining ones? We propose that this selectivity could be due to differential biochemical information recognised by the IFT components such as tubulin post-translational modifications. In the *T. brucei* flagellum, the most striking one is poly-glutamylation, the addition of glutamates to the C-terminal tail of tubulin that is catalyzed by TTLL (Tubulin Tyrosine Ligase-Like) enzymes. The opposite reaction is catalyzed by CCPs (Cytosolic CarboxyPeptidase). Using immunogold labelling, we showed that doublets 4 and 7 are differentially polyglutamylated. To evaluate the role of tubulin polyglutamylation on IFT, we have proceeded to overexpression of CCPs or down-regulation TTLLs in a tetracycline-dependent manner. When CCPs were overexpressed, a strong reduction of poly-glutamylation was observed on the cell body and in both mature and new flagella, whereas in knock-downed TTLL cells, the reduction of poly-glutamylation was observed mostly in the new flagellum. In both cases, IFT trafficking was impaired with accumulation of IFT proteins at the base of flagellum and reduced presence of IFT trains in the flagellum. These data reveal that selective tubulin poly-glutamylation confers a specific molecular identity to microtubules that is especially important for intraflagellar transport.

6E A molecular model of the mitochondrial genome segregation machinery in Trypanosoma brucei

Hoffmann, Anneliese (University of Bern, USA); Käser, Sandro (University of Bern, USA); Schneider, André (University of Bern, USA); Ochsenreiter, Torsten (University of Bern, USA)

The question of how eukaryotic cells ensure proper replication and maintenance of their organelles genome is of general interest in biology. *T. brucei* is the perfect model organism to approach this question. The parasite has, in comparison to mammalian or yeast, only one mitochondrion with one mitochondrial genome per cell and thus replication is tightly integrated in the cell cycle.

An electron microscopy study by Ogbadoyi and co-workers identified a structure they named tripartite attachment complex (TAC). They proposed this to be a physical connection between the basal body and the mitochondrial genome (kinetoplast DNA, kDNA). Others and we have now described six different proteins and a monoclonal antibody from three different regions of the TAC at varying levels of detail. Knockdown of any of those proteins leads mainly to a loss of kDNA or in some cells to enlarged kDNAs, followed by growth retardation.

Here we describe the *de novo* assembly of the two membrane-spanning complex by using RNAi and fluorescence microscopy. In addition, we show that the TAC assembly is carried out irrespective of the presence of the kDNA. We showed this by releasing RNAi after induction. Furthermore, using blue native gel electrophoresis in combination with western blotting we discovered that five of the currently known TAC components can be found in separate sub-complexes between 230kDa and >600kDa, indicating the enormous size of TAC and suggesting that many more proteins await discovery.

6F Controlling transferrin receptor trafficking with GPI-valence in bloodstream stage African trypanosomes

Tiengwe, Calvin (University at Buffalo (SUNY), Buffalo, NY, USA); Bush, Peter J. (University at Buffalo (SUNY), USA); Bangs, James D. (University at Buffalo (SUNY), USA)

Bloodstream-form African trypanosomes encode two structurally related GPI-anchored proteins that are critical virulence factors, variant surface glycoprotein (VSG) for antigenic variation and transferrin receptor (TfR) for iron acquisition. Both are transcribed from the active telomeric expression site. VSG is a GPI^2 homodimer; TfR is a GPI^1 heterodimer of GPI-anchored ESAG6 (E6) and ESAG7 (E7). We have previously shown that GPI-valence correlates with secretory progression and fate in bloodstream trypanosomes: VSG (GPI^2) is a surface protein; truncated VSG (GPI^0) is degraded in the lysosome; and native TfR (GPI^1) localizes in the flagellar pocket. Tf:Fe starvation results in up-regulation and redistribution of TfR to the plasma membrane suggesting a saturable mechanism for flagellar pocket retention. However, because surface TfR is non-functional we proposed that it represents GPI^2 E6 homodimers that are unable to bind transferrin - thereby mimicking native VSG [*J. Cell Sci*. (2005) 118:5499]. We now exploit a novel RNAi system for simultaneous lethal silencing of native TfR subunits and exclusive in-situ expression of RNAi-resistant ($RNAi^R$) TfR variants with valences of GPI^{0-2}. Our results conform to the valence model: GPI^0 E7 homodimers traffick to the lysosome and GPI^2 E6 homodimers to the cell surface. However, E6 alone is up-regulated, leaving the issue of saturable retention in play. Therefore, we created an $RNAi^R$ GPI^2 TfR heterodimer by fusing the C-terminal domain of E6 to E7 ($E7^{GPI}$). Co-expression with E6 generates a functional heterodimeric GPI^2 TfR that restores Tf uptake and viability, and localizes to the cell surface, without overexpression. These results resolve a longstanding controversy about TfR trafficking and confirm GPI valence as a critical determinant of intracellular sorting in trypanosomes.

SESSION VII: Molecular Biology
Vivian Bellofatto, Chair.
Wednesday 8:45 am

04/26/17

7A Role of long non-coding RNA in Trypanosoma brucei parasite

Guegan, Fabien (Instituto de Medicina Molecular, USA); Bento, Fabio (Intituto de Medicina Molecular, USA); Neves, Daniel (Instituto de Medicina Molecular, USA); Manso, Helena (Instituto de Medicina Molecular, USA); Figueiredo, Luisa (Instituto de Medicina Molecular, USA)

Trypanosoma brucei has a complex life cycle, which requires adaptation to different environments by remodeling gene expression. However, the molecular mechanism underlying this process is still not completely understood.

The advent of sensitive, unbiased, and high-throughput genomic technologies such as next-generation sequencing (NGS) revealed that, in all kingdoms of life, a large fraction of the transcribed genome is in the form of noncoding RNAs. Long non-coding RNAs (lncRNA) are a class of non-coding transcribed genes that are greater than 200 nucleotides and have emerged as master regulators of gene expression.

We have generated RNAseq data of *T. brucei* parasites that reveal the existence of hundreds of spliced and polyadenylated lncRNAs. *T. brucei* lncRNAs show similar characteristics to lncRNAs in other eukaryotes including their genomic location, length and GC content. Moreover, we identified 272 lncRNAs that are significantly differentially expressed between mammalian and insect stage of *T. brucei*, suggesting they may be involved in stage-specific gene regulation. RNA-FISH and Circularized RT-PCR assays confirmed the expression of a subset of these lncRNAs and revealed their nuclear localization. Finally, we identified one lncRNA that, upon overexpression, facilitates parasite differentiation from bloodstream slender forms towards the transmissible and quiescent stumpy forms.

Altogether, our work identified and characterized for the first time lcnRNA genes in *T. brucei* and it revealed a functional role for one of these molecules. Our results indicate that differentiation is under the control of non-coding RNAs.

7B RBP10 is another RNA-binding protein with a key role in the developmental progression to infectivity in Trypanosoma brucei

Shi, Huafang (Yale School of Public Health, USA); Ramey-Butler, Kiantra (Yale School of Public Health, USA); Christiano, Romain (Department of Cell Biology, School of Medicine, Yale University, USA); G Kolev, Nikolay (Yale School of Public Health, USA); C. Walther, Tobias (Department of Cell Biology, School of Medicine, Yale University, USA); Tschudi, Christian (Department of Epidemiology of Microbial Diseases, Yale School of Public Health, USA)

We previously established an *in vitro* differentiation system based on the overexpression of the RNA binding protein 6 (RBP6), which recapitulates many aspects of *Trypanosoma brucei* differentiation in the tsetse vector from procyclics to infectious metacyclics. We have used iCLIP (Individual-nucleotide resolution CrossLinking and ImmunoPrecipitation) to generate a transcriptome-wide biochemical footprint of RBP6-RNA interactions in living cells at 24 hours after induction of RBP6 expression to capture the primary and likely critical mRNA targets. Among the 526 mapped interactions of RBP6 with mRNAs with the consensus 5'-TN[T/A]TCC-3', two binding sites were found in the 3' UTR of the RBP10 mRNA, suggesting that RBP6 has a direct role in the regulation of RBP10 expression. Interestingly, this protein was shown previously (Wurst *et al*., 2012) to be able to remodel the *T. brucei* transcriptome from a procyclic to a more "bloodstream-form pattern". In addition, transcriptomic and proteomic analysis of metacyclics revealed RBP10 as the most up-regulated RNA-binding protein as compared to procyclics, with a 16- and 5-fold up-regulation at the protein and mRNA level, respectively. RNAi-induced down-regulation of RBP10, in the background of inducible expressing of RBP6 resulted in the lack of production of metacyclics. Similarly, knock-out of RBP10 confirmed the RNAi results and also showed that no metacyclic VSGs were expressed. We further showed that 2 kb of the RBP10 3'UTR, containing the two iCLIPs, can drive RBP6-dependent expression of a reporter mCherry construct.

7C Control of allelic exclusion by a trypanosome 'Vex Histone Chaperone' complex

Faria, Joana C. (School of Life Sciences, University of Dundee, Dundee, GBR); Glover, Lucy (Institut Pasteur, USA); Hutchinson, Sebastian (Institut Pasteur, USA); Boehm, Cordola (School of Life Sciences, University of Dundee, USA); Field, Mark C. (School of Life Sciences, University of Dundee, USA); Horn, David (School of Life Sciences, University of Dundee, USA)

Specialised metazoan cells and pathogenic protozoa can activate a single gene from a family of closely related genes, but the underlying mechanisms have remained mysterious. In parasitic trypanosomes, association of one telomeric variant surface glycoprotein (*VSG*) gene with an RNA-polymerase-I (pol-I) transcription factory known as the expression-site body (ESB), and with VSG exclusion 1 (VEX1), facilitates monotelomeric *VSG* expression and antigenic variation. To probe the mechanism of *VSG* allelic exclusion, we isolated VEX1-interactors and used quantitative proteomics to identify them. We found that a Vex Histone Chaperone or 'VHC' complex incorporates a putative RNA helicase (VEX2) and the conserved replication-associated histone chaperone, chromatin assembly factor 1 (CAF-1). VEX2 forms a single sub-nuclear focus that colocalises with VEX1, immediately adjacent to the ESB, and CAF-1 displays enrichment in the same nuclear compartment, particularly in S-phase. VEX1 displays the properties of a limiting transcription factor that activates pol-I transcription and becomes sequestered as a result. Specifically, all known pol-I transcribed genes are activated when VEX1 is overexpressed or when VEX2 is knocked down, which causes VEX1 redistribution. In addition, transcription inhibition leads to redistribution of VEX1 and VEX2 to multiple sub-nuclear foci. VEX2 mediates post-transcriptional suppression of other Expression Site Associated Genes (*ESAGs*), boosting the proportional output from the *VSG*, while CAF-1 blocks transcription at other telomeres. In summary, a VHC complex activates one telomeric *VSG* and excludes others through a mechanism involving locus-specific sequestration of a transcription factor and histone-chaperone dependent silencing of other alleles. Our results provide a new "winner-takes-all" paradigm for the establishment and inheritance of allele-specific epigenetic states.

7D The ZC3H39/40 RNA-binding complex and control of an electron transport chain regulome in African trypanosomes

Trenaman, Anna (University of Dundee, Dundee, GBR); Hutchinson, Sebastian (Institut Pasteur, USA); Glover, Lucy (Institut Pasteur, USA); Horn, David (University of Dundee, USA)

We ran an RNAi screen in bloodstream form *T. brucei* that implicated the RNA-binding proteins, ZC3H39 and ZC3H40 in variant surface glycoprotein (VSG) gene silencing. Knockdown of either protein disrupted VSG silencing as determined by western blotting, flow-cytometry and RNA-seq. The presence of a cytoplasmic ZC3H39/40 complex in *T. brucei* was supported by immunofluorescence co-localisation, co-destabilisation and co-immunoprecipitation. A CLIP-seq experiment, surprisingly, identified transcripts encoding multiple components of the electron transport chain, including those for cytochrome oxidase and the F-ATPase. Consistent with this association, these same transcripts were down-regulated in ZC3H39/40 knockdowns as determined by RNA-seq and by stable isotope labelling in cell culture followed by mass-spectrometry and proteomic profiling. Notably, this regulation was only observed in bloodstream form parasites following ZC3H40 knockdown. Thus, we report an RNA-binding complex that controls the expression of electron transport chain components in *T. brucei*. Our findings also establish an intriguing link between an oxidative phosphorylation control electron transport chain regulome and VSG expression control.

7E Deep analysis of tails on Trypanosoma brucei mitochondrial transcripts reveals life stage as well as transcript-specific regulation

Zimmer, Sara L. (University of Minnesota Medical School, Duluth, MN, USA); Gazestani, Vahid (McGill University Institute of Parasitology, USA); Hampton, Marshall (University of Minnesota Duluth, USA); Shaw, Aubie (University of Minnesota Medical School, USA); Kalem, Murat (University of Minnesota Duluth, USA); Liggett, Charles (University of Minnesota, USA); Salavati, Reza (McGill Institute of Parasitology, USA)

Trypanosoma brucei parasites are exposed to extreme changes in available nutrients and other factors during their life cycle. Thus regulation of mitochondrial genes is necessary for adaptation and survival. However, mechanisms of developmental regulation of mitochondrial genes, comprehensively profiled here at the RNA level, are incompletely understood. 3' non-templated nucleotide tails consisting of adenine and uridine on mitochondrial transcripts play roles in gene expression regulation. Theoretically they could also facilitate this developmental regulation of mitochondrial gene expression. *T. brucei* mitochondrial transcripts can possess two compositionally distinct tail states, each with a differing functional role; i.e., while one tail type is implicated in RNA stability (in-tails), the other type appears associated with translation (ex-tails). We utilized circTAIL-seq to examine existence of life stage differences between tail populations, a crucial step towards exploring developmental tail regulatory roles. Our results provide the first evidence demonstrating longer in-tails in the mammalian life stage for CO1, ND1, and CO3 transcripts, and also show stage-specific differences in 5' and 3' UTR lengths. We also developed methodology to systematically distinguish different tail states, by which we demonstrated expected depletion of ex-tailed CO1 in mammalian stage cells where CO1 is destabilized. Our findings suggest that non-encoded tails are implicated in the developmental modulation of mitochondrial transcripts.

7F Toward the determination of spindle-associated proteome in Trypanosoma brucei

Lee, Kyu j. (University of Texas, houston, TX, USA); Zhou, Qing (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA); Hu, Huiqing (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA); Li, Ziyin (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA)

The faithful distribution of sister chromatids to daughter cells during cell division, which is carried out by the mitotic spindle, is crucial to maintain genome integrity. *Trypanosoma brucei* assembles a mitotic spindle inside the nuclear envelope in the absence of centrosomes, and lacks the spindle motor BimC and many conserved spindle-associated proteins. To understand the mechanism of spindle assembly and the regulation of chromosome segregation in *T. brucei*, we attempt to identify spindle-associated proteins and determine their roles in chromosome segregation. Through BioID and mass spectrometry, we have identified 22 spindle-associated proteins, all of which are kinetoplastid-specific proteins. Intriguingly, 20 of these 22 proteins localize to the nucleolus during interphase and to the spindle during mitosis. We named these proteins NOSAP for nucleolar and spindle associated proteins. The other two proteins localize to spindle poles during mitosis and to the nucleus during interphase and the spindle during mitosis, respectively, and were named SPB1 (spindle pole body 1) and NUSAP1 (nuclear and spindle associated protein 1). BioID with certain spindle-associated proteins also identified some kinetochore proteins as near neighbors/binding partners, suggesting their potential roles in mediating spindle-kinetochore interactions. To understand the physiological functions of these spindle-associated proteins, RNAi of the representative NOSAP proteins, SPB1 and NUSAP1 was performed, and depletion of these proteins caused distinct defects in chromosome segregation and nucleus morphology. Collectively, these results identified novel spindle-associated proteins, detected the proteins that locate at the spindle-kinetochore interface, and revealed distinct roles of these proteins in chromosome segregation in *T. brucei*. This work laid the foundation for understanding of spindle assembly, chromosome segregation and spindle-kinetochore interaction in *T. brucei*.

7G Kinetoplastid Ndc80/Nuf2 reunites chromosome segregation across eukaryotes

D'Archivio, Simon (University of Nottingham, USA); Wickstead, Bill (University of Nottingham, USA)

Kinetochores are multi-protein complexes that couple eukaryotic chromosomes to the mitotic spindle to ensure proper segregation. A paradigm exists for kinetochore composition and function, but key components are apparently absent in some lineages. In kinetoplastids, none of the widely conserved subunits could be readily identified as encoded in the completed genome and the recent identification of unconventional kinetochore proteins with no apparent homology to model organisms suggested that more than one system for eukaryotic chromosome segregation may exist. Here, we report a new family of proteins distantly related to the outer kinetochore proteins Ndc80 and Nuf2. The family member in *Trypanosoma brucei*, KKIP1, associates with the kinetochore and its depletion causes severe defects in karyokinesis, loss of individual chromosomes, and gross defects in spindle assembly or stability. Immuno-purification of KKIP1 from stabilized kinetochores identifies 6 further components, which form part of a trypanosome outer kinetochore complex. Thus, KKIP1 has detectable similarity to Ndc80/Nuf2, phenocopies aspects of Nuf2 mutants, and sits at the same position relative to inner kinetochore components as is seen for Ndc80 in yeast or animal cells. These findings show that, although kinetochores in kinetoplastids are unusual in composition, they are not ancestrally distinct from those in other lineages and that Ndc80/Nuf2-like proteins are most likely universal in eukaryotic mitosis. It also suggests a method for detection of deep homologues that might substantially improve our ability to understand the biology of these parasites based on genome sequence.

POSTERS: Session A
Sunday 7:00 pm

04/23/17

1 FRET Flow Cytometry and Microscopy for Monitoring Glucose in Trypanosoma brucei

Christensen, Kenneth A. (Brigham Young University, Provo, UT, USA); Voyton, Charles (Clemson University, USA); Ackroyd, Christine (Brigham Young University, USA); Morris, Meredith (Clemson University, USA); Morris, James (Clemson University, USA)

Since the bloodstream form (BSF) of *Trypanosoma brucei* relies solely on ATP production via glucose metabolism in glycosomes, understanding how glucose is transported and regulated in the parasite is key for basic trypanosome biochemistry and developing therapeutic strategies. Currently, information about intracellular glucose has been limited by the difficulty of making measurements in organelles. We have utilized a non-destructive technique that adapts a fluorescence resonance energy transfer (FRET) protein biosensor (FLII12PGLu-600u) to monitor cytosolic and glycosomal glucose in live *T. brucei*. FLII12PGlu-600u localizes to the cytosol, allowing for measurement of FRET response to cytosolic glucose levels. Appending a type 1 peroxisomal targeting sequence (PTS1) to the biosensor (PTS1-FLII12PGlu-600u) results in glycosomal localization. The resulting sensors allow real-time monitoring of both cytosolic and glycosomal glucose concentration in live BSF and procyclic form (PCF) parasites. We have investigated the effect(s) of glucose metabolic inhibitors, changes in external glucose, monovalent cation concentrations, and pH on intracellular and glycosomal glucose concentrations. Notably, our data suggest that glycosomal glucose in PCF parasites is substantially higher than external levels, presumably reflecting the need of PCF parasites to compensate for low glucose conditions in the fly midgut. In contrast, glycosomal glucose levels in BSF parasites are indistinguishable from external levels, but may be enriched relative to the cytosol. These differences between BSF and PCF trypanosomes support evolutionary adaptation together with active glucose transport mechanisms. While we have focused here on glucose, a large repertoire of available FRET sensors can be similarly adapted for measurement additional key cellular analytes in *T. brucei* and other kinetoplastids.

2 Variant antigen profiling: a tool for genome-wide analysis of Variant Surface Glycoprotein diversity in Trypanosoma congolense

Silva Pereira, Sara (University of Liverpool, Liverpool, GBR); Casas, Aitor (Liverpool School of Tropical Hygiene, USA); Haines, Lee (Liverpool School of Tropical Medicine, USA); Noyes, Harry (University of Liverpool, USA); Sanders, Mandy (Wellcome Trust Sanger Institute, USA); Berriman, Matthew (Wellcome Trust Sanger Institute, USA); Acosta-Serrano, Alvaro (Liverpool School of Tropical Medicine, USA); Jackson, Andrew (University of Liverpool, USA)

Antigen variation is the mechanism through which African trypanosomes modulate the Variant Surface Glycoprotein (VSG) comprising their cell surface coat, resulting in chronic infection. Periodic switching of the expressed VSG, coupled with the host immune response, causes sequential expansion of parasite clones each expressing a serologically distinct VSG. Genome sequencing has revealed hundreds of VSG genes and this presents challenges for analyzing VSG dynamics over space and time. We have developed a method for analyzing VSG diversity in *Trypanosoma congolense* on a population scale using high-throughput sequencing data.

Previously, we showed that *T. congolense* VSGs segregate into defined 'phylotypes', between which there is no evidence for recombination. Using forty *T. congolense* clinical isolates from six African countries, we have discovered that these phylotypes are universal and exhaustive. Exploiting this predictable repertoire, we developed the 'Variant Antigen Profile' (VAP) as a metric of VSG diversity. The VAP calculates the relative frequencies of distinct VSG 'phylotypes' from genomic or transcriptomic data, to rapidly describe the repertoire in a given strain or condition.

To demonstrate, we applied variant antigen profiling to VSG expression in metacyclic cells of *T. congolense* strain 1/148 in the tsetse fly. Transcriptomic and proteomic analyses of both pooled and individual fly mouthparts show that the profile of VSG expression is both reproducible and non-random, suggesting that certain phylotypes may be preferentially expressed in metacyclics.

Together, our results show that the VAP is useful for quantifying VSG diversity from sequence data, without need for annotation. We expect this to facilitate studies of antigenic diversity in natural populations, and to extend our understanding of the functional consequences of VSG structural diversity.

3 Trypanosoma brucei RAP1 suppresses VSG switching by repression of telomere transcription and telomere RNA:DNA hybrids

Nanavaty, Vishal P. (Cleveland State University, Center for Gene Regulation in Health and Disease, Cleveland, OH, USA); Sandhu, Ranjodh (University of California Davis, USA); Jehi, Sanaa (The Scripps Research Institute, USA); Pandya, Unnati (New York University, School of Medicine, USA); Li, Bibo (Cleveland State University, Center for Gene Regulation in Health and Disease, USA)

Antigenic variation is an obligatory mechanism for a long-term survival of *Trypanosoma brucei* inside its mammalian host. *T. brucei* expresses its major surface antigen, VSG, from one of the fifteen subtelomeric expression sites in a strictly monoallelic manner and regularly switches its VSG coat to evade the host immune response. It has been shown that telomeres and telomeric proteins play important roles in VSG silencing and VSG switching regulation. VSG switching can be transcriptional (*in situ* switching) or DNA recombination-mediated (such as gene conversion and reciprocal telomeric crossover). Previously we have reported that *Tb*RAP1, an essential telomeric protein in *T. brucei*, is required for VSG silencing and suppresses VSG gene conversion-mediated switching. Surprisingly, in *Tb*RAP1-depleted cells we also observed an increased level of the telomeric transcript, TERRA, and an increase in telomeric RNA:DNA hybrids (R-loops). In other organisms, R-loop has been shown to induce DNA double strand breaks (DSBs), while a single DSB in the active ES is detrimental to the survival of *T. brucei*. Here we report that, depletion of *Tb*RAP1 also increased the amount of DSBs in the active and silent ESs. R-loops are sensitive to RNAseH, a ribonuclease that degrades the RNA strand of the RNA:DNA hybrid. Ectopic expression of *Tb*RNaseH1 in *Tb*RAP1 RNAi cells resulted in reduction of the number of telomeric R-loops back to the WT level and suppressed the elevated VSG switching frequency phenotype. We also detected a normal level of DSBs at the active and silent ESs in *Tb*RNaseH1-expressing, *Tb*RAP1-depleted cells. Therefore, we propose that more telomeric R-loops in *Tb*RAP1-depleted cells promotes DSB-induced VSG gene conversion-mediated switching, and *Tb*RAP1-depletion results in a defective telomere/subtelomere integrity maintenance that leads to cell growth arrest.

4 Puzzling together the Trypanosoma brucei Lister 427 genome

Cosentino, Raúl O. (Research Center for Infectious Diseases, University of Würzburg, Würzburg, DEU); Müller, Laura SM (Center for Infectious Disease Research, University of Würzburg, USA); Förstner, Konrad U. (Core Unit Systems Medicine, University of Würzburg, USA); Lajoie, Bryan R. (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, USA); Kaplan, Noam (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, USA); Belton, Jon-Matthew (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, USA); Steinbiss, Sascha (Parasite Genomics, Wellcome Trust Sanger Institute, Hinxton, USA); Otto, Thomas D. (Parasite Genomics, Wellcome Trust Sanger Institute, Hinxton, USA); Berriman, Matthew (Parasite Genomics, Wellcome Trust Sanger Institute, Hinxton, USA); Sebra, Robert P. (Icahn Institute and Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, USA); Dekker, Job (Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA); Siegel, T. Nicolai (Research Center for Infectious Diseases, University of Würzburg, USA)

Although Lister 427 is the most widely used strain in the *T. brucei* field, only the genome of the TREU 927 strain is fully assembled and serves as reference genome.

Previous studies have revealed that the content and extension of the subtelomeric VSG arrays is completely different between these strains, being much larger in the Lister 427 strain.

The lack of a complete Lister 427 genome, or a suitable reference, has made 'true' genome-wide analyses impossible, especially regarding the analysis of antigenic variation related phenomena, highlighting the importance of obtaining a complete Lister 427 strain genome sequence. However, the generation of complete and accurate trypanosome genome assemblies has been hindered by the high amount of repetitive sequence elements present in their genomes.

To overcome this problem, we combined long read sequencing technology (PacBio sequencing) with DNA-DNA contact data obtained from genome-wide chromosome conformation capture (Hi-C) experiments. We exploited strong ubiquitous features of 3D-architecture of eukaryotic genomes, to order and scaffold the assembled fragments.

The resulting scaffold of the eleven chromosome genome has a size of ~42 Mb, 30% larger than the reference genome. Most of the additional DNA content represents extension of the VSG repertoire. We found that VSGs are arranged in extremely long hemizygous subtelomeric arrays, in some cases longer than the homozygous core region. In addition, we were able to determine the location of many known VSG expression sites and identify new putative ones.

The availability of the Lister 427 genome assembly with complete VSG repertoire will provide a better platform on which to study gene function and variation in *T. brucei*.

5 Biochemical and Genomic Analysis of MRB10130 Function in RNA Editing

Tylec, Brianna (University at Buffalo, USA); Simpson, Rachel (University at Buffalo, USA); Harrison, Gregory (University at Buffalo, USA); Chen, Runpu (University at Buffalo, USA); Sun, Yijun (University at Buffalo, USA); Read, Laurie (University at Buffalo, USA)

Uridine (U) insertion/deletion editing of select mitochondrial mRNAs is an essential process in kinetoplastids whereby mRNA is modified to generate a translatable open reading frame with the use of *trans*-acting guide RNAs (gRNAs). gRNAs are sequentially utilized as templates, which ensures the general 3' to 5' progression of editing along the mRNA. This process involves multiple protein subcomplexes including the non-enzymatic RNA Editing Substrate Binding Complex (RESC), composed of the RNA Editing Mediator Complex (REMC) and Guide RNA Binding Complex (GRBC). The protein MRB10130 has been shown to have interactions with the REMC and GRBC subcomplexes and is essential for the editing process and for growth. Pulldowns in the presence and absence of MRB10130 show that it is needed for the interaction of REMC with GRBC and vice versa. The exact mechanism by which MRB10130 mediates editing is yet unclear. To define the role of MRB10130 in editing initiation and progression, we used high throughout sequencing and the Trypanosome RNA Editing Alignment Tool (TREAT) to investigate the effects of MRB10130 on both pan-edited and minimally edited transcripts. We examine the effects of MRB10130 knockdown on RNA editing initiation and progression. We then compare these to the effects of knockdowns of REMC component TbRGG2, known to affect the progression of editing through gRNAs, and GRBC components MRB3010 and GAP1, involved in editing initiation.

6 A protein kinase inhibitor-based genome-wide RNAi screen to identify regulators of stumpy formation in Trypanosoma brucei

MacDonald, Lindsay (University Of Edinburgh, USA); Cayla, Mathieu (University Of Edinburgh, USA); Mony, Binny (University Of Edinburgh, USA); MacGregor, Paula (Uiversity of Cambridge, USA); Navarro, Miguel (Instituto de Parasitología y Biomedicina "López-Neyra", USA); Matthews, Keith R. (University of Edinburgh, Edinburgh, GBR)

A recent high-throughput screen using the GlaxoSmithKline kinase inhibitor set (Diaz, et al; PMID: 25340575) successfully identified several hundred compounds that prevent the growth of *T. brucei* in vitro. Although most would target essential processes, leading to parasite stasis or death, we anticipated a subset might generate their growth effect through promoting the differentiation of parasites to arrested stumpy forms. Therefore, 52 inhibitors for which structure was available were tested on a *T. brucei* pleomorphic reporter line, in which development to stumpy forms would activate the expression of GUS regulated by the 3'UTR of the stumpy-specific PAD1 gene. This identified 6 compounds able to activate GUS to at least 50% of the activation level generated by pCPT-cAMP, known to mimic stumpy formation in vitro. Of these, one compound, GKI7, activated GUS at an equivalent level to the positive control. To validate GKI7 further, its activity against null mutant lines for several components of the stumpy induction factor signalling pathway was tested. This demonstrated that knockout of RBP7, previously shown to be required for stumpy formation, rendered parasites resistant to GKI7 demonstrating action of the inhibitor on this pathway. A genome-wide RNAi screen was then performed to identify molecules whose silencing rendered parasites resistant to GKI7. The selected parasites were subject to RNAi insert identification by ion-torrent sequencing, this identifying RBP7 as one target, validating the screen. In addition, a further set of ~20 genes were identified that had not been previously identified in developmental screens. These molecules are currently undergoing validation with respect to their involvement in GKI7 sensitivity in vitro and slender to stumpy differentiation in vivo.

7 Endosymbiosis, origins and gene expression in the photosynthetic protist Euglena gracilis

Ebenezer, ThankGod (Department of Biochemistry, University of Cambridge, Cambridge, GBR); Nenarokova, Anna (Department of Parasitology, Charles University in Prague, USA); Vanclová, Anna (Biology Centre, Institute of Parasitology, Czech Academy of Sciences, USA); Zoltner, Martin (School of Life Sciences, University of Dundee, USA); Obado, Samuel (Laboratory of Cellular and Structural Biology, The Rockefeller University, USA); Santana, Carlos (Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, USA); Devos, Damien (Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, USA); Hampl, Vladimir (Department of Parasitology, Charles University in Prague, USA); Ginger, Michael (Department of Chemical & Biological Sciences, School of Applied Sciences, University of Huddersfield, USA); Lukes, Julius (Biology Centre, Institute of Parasitology, Czech Academy of Sciences, USA); Dacks, Joel (Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta, USA); Carrington, Mark (Department of Biochemistry, University of Cambridge, USA); Kelly, Steven (Department of Plant Sciences, University of Oxford, USA); Field, Mark (School of Life Sciences, University of Dundee, USA)

The photosynthetic flagellate *Euglena gracili*s harbours a secondary endosymbiotic plastid and is a distant relative of pathogenic trypanosomatids, a major component of global aquatic ecosystems and of considerable biotechnological potential with resistance to harsh conditions. Here we report genome, transcriptome and proteome drafts for *E. gracilis*. The genome is over 2Gb and has a coding potential of 36,526 predicted ORFs. Less than 25% of the genome is single copy sequence, indicating extensive repeat elements. Several gene families likely associated with the cell surface and signal transduction possess very large numbers of lineage-specific paralogs, suggesting great flexibility in environmental monitoring and, together with divergent mechanisms for metabolic control, novel solutions to adaptation to extreme environments. There are clear contributions from photosynthetic eukaryotes to the nuclear genome with red, green and brown algael genes evident, together with orthogroups shared with only trypanosomes and also with other excavates. Furthermore, we demonstrate that the majority of control of protein expression level is post-transcriptional despite the presence of conventional introns, that mRNA metabolism is highly unusual in transcriptional and nuclear export mechanisms and which differentiate Euglenids from the trypanosomatids. These data are a major advance in the understanding of the nuclear genome of Euglenids and provide a platform for investigation of the contributions of *Euglena gracilis* and relatives to the biosphere.

8 RNA Editing Mediator Complex proteins have distinct functions in gRNA utilization and reveal the essentiality of non-contiguous editing modifications

Simpson, Rachel (Department of Microbiology & Immunology, University at Buffalo Jacobs School of Medicine, Buffalo, NY 14214, Buffalo, NY, USA); Bruno, Andrew (Center for Computational Research, University at Buffalo, USA); Chen, Runpu (Department of Computer Science and Engineering, University at Buffalo, USA); Lott, Kaylen (Department of Microbiology and Immunology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, USA); Tylec, Brianna (Deparment of Microbiology and Immunology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, USA); Bard, Jonathan (Genomics and Bioinformatics Core, University at Buffalo, USA); Sun, Yijun (Deparment of Microbiology and Immunology and Department of Computer Science and Engineering, University at Buffalo Jacobs School of Medicine and Biome, USA); Buck, Michael (Department of Biochemistry, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, USA); Read, Laurie (Department of Microbiology and Immunology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, USA)

Uridine insertion/deletion RNA editing is an essential process in kinetoplastids whereby mitochondrial mRNAs are modified by specific insertion and deletion of uridines. Editing generates functional open reading frames that encode mitochondrial respiratory proteins. Both enzymatic and non-enzymatic factors are required for RNA editing. The roles numerous non-enzymatic factors have remained opaque given the limitations of conventional methods to interrogate the order and mechanism by which editing progresses and thus the roles individual proteins play in mediating this progression. To overcome this limitation, we developed a novel bioinformatic platform, the Trypanosome RNA Editing Alignment Tool (TREAT), which allows us to examine whole populations of partially edited sequences using high throughput sequencing (HTS). Using HTS/TREAT, we elucidated the roles of three proteins in the RNA Editing Mediator Complex (REMC). We determined that the three factors examined function in the progression of editing through a gRNA, rather than in gRNA exchange. However, they have distinct roles, and REMC is likely heterogeneous in composition. MRB8170/4160 plays a transcript-specific role in editing initiation, and constrains the region of active editing. In contrast, TbRGG2 and MRB8180 are required for editing to proceed through a gRNA-defined region in a manner that does not go strictly site-by-site (*i.e.*, non-linear), presumably due to their ability to promote gRNA/mRNA plasticity. We provide the first evidence that editing can proceed through distinct pathways and that non-linear modifications are essential to the editing process, generating commonly observed junction regions. Our data support a model in which RNA editing is executed via multiple pathways that necessitate successive re-modification of junction regions facilitated, in part, by the REMC variant containing TbRGG2 and MRB8180.

9 The 5S ribonucleoprotein particle (5S RNP) assembly factor protein, Rpf2, is an essential protein in Trypanosoma brucei

Kamina, Anyango (University at Buffalo, Buffalo, NY, USA); Williams, Noreen (USA)

The assembly of the 5S ribonucleoprotein particle (5S RNP) into the 60S subunit functions as a regulator of ribosome biogenesis a conserved and essential cellular process in all organisms. In eukaryotes, the nucleolar 5S RNP is composed of 5S ribosomal RNA (rRNA), ribosomal proteins L5 and L11, and assembly factors Rpf2 and Rrs1. We have already shown that the *T. brucei* nucleolar 5S RNP is composed of 5S rRNA, L5, and trypanosome-specific RNA binding proteins P34 and P37. To determine whether the other components are present, we performed tandem affinity purification using extracts from PTP tagged cell lines. We identified the *T. brucei* Rpf2 homolog using mass spectrometry analysis.

This is the first study to characterize the Rpf2 protein in an organism other than fungi. Induction of RNAi knockdown of TbRpf2 leads to rapid cell death and causes defects in ribosome formation. We observed an accumulation of 40S subunit, a decrease in the formation of the 60S subunit and a decrease in polysome formation. *In vitro*, Yeast Rpf2 is more stable and functional when expressed as an Rpf2-Rrs1 complex. We have successfully purified a functional and stable recombinant TbRpf2 protein in the absence of Rrs1. We have shown that TbRpf2 forms conserved interactions with L5, and 5S rRNA and that it forms trypanosome-specific interactions with P34 and P37. These studies not only provide a better understanding of ribosome biogenesis but also allow for the characterization of trypanosome-specific interactions that can potentially function as new therapeutic targets.

10 Flagellum Attachment Zone and the control of Leishmania flagellar pocket shape in both promastigotes and amastigotes

Sunter, Jack D. (Oxford Brookes University, Oxford, GBR); Yanase, Ryuji (University of Hyogo, USA); Moreira-Leite, Flavia (University of Oxford, USA); Gull, Keith (University of Oxford, USA)

The flagellar pocket (FP) of *Leishmania* is an essential conduit through which the cell interacts with the environment and is critical for pathogenicity. In *Leishmania* promastigotes the flagellum is described as "free", extending from the FP at the cell anterior; yet these parasites have homologs of many flagellum attachment zone (FAZ) proteins, which in trypanosomes attach the flagellum laterally to the cell body. We have previously shown that *Leishmania* have a FAZ structure, which is intrinsically linked to the morphogenesis of the FP, implicating the FAZ in *Leishmania* cellular design for pathogenicity.

Here, using a gene knockout strategy we investigated the function of the FAZ in *Leishmania*. LmFAZ5 knockout in promastigotes disrupts the organisation of the FAZ structure resulting in a reduction in cell size. Electron microscopy of these cells shows a loss of attachment between the flagellum and the cell body, resulting in changes to the shape and size of the FP/neck region. In LmFAZ5 knockout axenic amastigotes the loss of attachment in the neck region results in a dramatically different FP/neck organisation and an increase in the length of flagellum outside the cell body. These data show that the FAZ in *Leishmania* is critical for determining cell shape and size and crucially for maintaining the shape of the FP and neck regions in both promastigotes and amastigotes, potentially impacting on both vector and host cell infectivity.

11 MRB7260 is essential for productive protein-RNA interactions during RNA editing in Trypanosoma brucei

McAdams, Natalie (SUNY Buffalo, USA); Simpson, Rachel (SUNY Buffalo, USA); Read, Laurie (SUNY Buffalo, USA)

Mitochondrial RNA editing in *T. brucei* is a unique process that involves the proper association of many RNAs and proteins. This uridine insertion/deletion RNA editing is catalyzed by RECC (RNA editing core complex; a.k.a. 20S editosome), which contains endonuclease cleavage, uridine insertion/deletion, and ligase activities, and directed by small trans-acting guide RNAs (gRNAs). In recent years, the RNA Editing Substrate Binding Complex (RESC) has come to the forefront as another vital player in mitochondrial RNA editing. RESC is comprised of at least two subcomplexes: GRBC (Guide RNA Binding Complex) and REMC (RNA Editing Mediator Complex). Together, RESC serves as the platform for RNA editing and coordinates interactions between RECC, mRNA, and gRNA. We recently characterized an additional RESC protein, MRB7260 (Tb927.9.7260), which we show is essential for *T. brucei* cell growth and mitochondrial RNA editing. MRB7260 lacks RNA binding activity, and is present in assembled RESC complexes as well as in smaller complexes containing GAP1/2, a component of GRBC that is essential for gRNA stabilization, and REMC protein, MRB8170. RNAi studies show that MRB7260 promotes the association between GRBC and REMC subcomplexes. Moreover, it is essential for proper gRNA and mRNA trafficking within RESC. Deep sequencing analysis of partially edited mRNAs in MRB7260-depleted cells indicates that the protein facilitates gRNA utilization, with the exception of the initiating gRNA, and is thus required for the 3' to 5' progression of editing. In sum, our studies identify MRB7260 as a protein essential for productive association of protein and RNA during uridine insertion/deletion RNA editing.

12 Transcriptomes of Trypanosoma brucei rhodesiense from sleeping sickness patients, rodents and culture: effects of growth conditions and RNA preparation methods

Mulindwa, Julius (Makerere University, USA); Leiss, Kevin (Heidelberg University, USA); Ibberson, David (Heidelberg University, USA); Matthews, Keith (University of Edinburgh, USA); Matovu, Enock (Makerere University, USA); Enyaru, John (Makerere University, USA); Clayton, Christine (ZMBH, Heidelberg, DEU)

All of our current knowledge of African trypanosome metabolism is based on results from trypanosomes grown in culture or in rodents. Drugs against sleeping sickness must however treat trypanosomes in humans. We here compare the transcriptomes of *Trypanosoma brucei rhodesiense* from the blood and cerebrospinal fluid of human patients with those of trypanosomes from culture and rodents. The data were aligned and analysed using new user-friendly applications designed for Kinetoplastid RNA-Seq data. These applications will be demonstrated at the meeting.

The transcriptomes of trypanosomes from human blood and cerebrospinal fluid did not predict major metabolic differences that might affect drug susceptibility. Usefully, there were relatively few differences between the transcriptomes of trypanosomes from patients and those of monomorphic laboratory-adapted parasites grown in *in vitro* culture. However, comparisons across multiple datasets suggest that the RNA preparation method, as well as the parasite source, selectively affect the apparent abundances of mRNAs encoding ribosomal proteins and distinct subsets of protein kinases and RNA binding proteins.

13 Identifying Critical Interactions in the Unique Trypanosoma brucei 5S Ribonucleoprotein Complex and their Role in Ribosome Biogenesis

Jaremko, Daniel (SUNY University at Buffalo, Buffalo, NY, USA); Williams, Noreen (SUNY University at Buffalo, USA)

Trypanosoma brucei *gambiense* and *rhodesiense* are responsible for Human African Trypanosomiasis (HAT), which is nearly always fatal without treatment. However, current medications have numerous problems and are rife with adverse side effects. One approach to developing new drugs is targeting processes that are essential for survival and pathogen-specific. Ribosome biogenesis is the process of assembling protein and RNA components into ribosomes. One critical part is the formation and incorporation of the 5S ribonucleoprotein (RNP) complex into developing 60S ribosomal subunits, which is a regulatory checkpoint. Work in our laboratory has identified the trypanosome-specific proteins P34/P37 as an essential part of the *T. brucei* 5S RNP. We have also shown direct *in vitro* interactions between P34/P37 and the protein L5 and 5S rRNA, two well-studied components of the 5S RNP. Recently, we identified *T. brucei* homologues of the proteins L11, Rpf2 and Rrs1, which are involved in maturation and incorporation of the 5S RNP in yeast, and have identified unique properties of *T. brucei* Rpf2. Initial work with L11 has resulted in the purification of recombinant $(His)_6$-tagged protein and has shown direct binding to 5S rRNA to L11 with a K_D of 227 nM. Our future work will continue our analysis of L11, and will also examine Rrs1 on its own as well as part of an Rrs1/Rpf2 heterodimer, expanding on our laboratory's previous work with Rpf2. Mapping the network of interactions that occur in the unique context of the *T. brucei* 5S RNP will identify targets for future drug development.

14 Exploring regulatory mechanisms behind the function of a major T. brucei arginine methyltransferase

Kafkova, Lucie (Department of Microbiology and Immunology, Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of medicine, Buffalo, NY, Buffalo, NY, USA); Debler, Erik (Laboratory of Cell Biology, The Rockefeller University, New York, NY, USA); Read, Laurie (Department of Microbiology and Immunology, Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of medicine, Buffalo, NY, USA)

Protein arginine methyltransferases (PRMTs), enzymes that catalyze formation of methylarginines, are nearly ubiquitous and relatively conserved among eukaryotes. We study PRMTs in the early divergent parasitic protozoan, *Trypanosoma brucei*. In this organism, at least 15% of the proteome is decorated by arginine methylmarks, including proteins in numerous cellular compartments and with functions in wide-ranging processes such as RNA processing, DNA repair, metabolism, and protein trafficking. The majority of asymmetrically dimethylated arginine (ADMA) is catalyzed in vivo by the *T. brucei* homolog of human PRMT1, TbPRMT1.

We recently showed that TbPRMT1 functions as a heterotetrameric complex comprising of catalytically active subunit (enzyme) that is robustly activated by a catalytically dead PRMT paralog (prozyme). This is the first example of an enzyme-prozyme pair outside of the *T. brucei* polyamine synthesis pathway and a novel PRMT architecture in any organism. We are currently investigating two avenues of TbPRMT1 regulation: interaction with nucleic acids and post-translational modifications (PTMs). Proteome-wide studies reported that the prozyme, but not the enzyme, is phosphorylated, bound to mRNA, and trafficked to stress granules in starved trypanosomes. Our preliminary data show that the prozyme can indeed bind RNA both in vivo and in vitro, an interaction never before reported for PRMTs. This interaction could impact recruitment of TbPRMT1 to RNA binding proteins, which constitute the major group of proteins bearing arginine methylmarks, or play a role in *T. brucei* starvation stress response.

Furthermore, mutations mimicking reported prozyme phosphorylations substantially decrease complex formation between bacterially co-expressed TbPRMT1 subunits. We are exploring the possibility that phosphorylation of prozyme could be a signal leading to general decrease of TbPRMT1 activity.

15 Characterizing the nuclear export proteins TbMex67-TbMtr2 interactions with the 5S RNP component of the 60S ribosomal subunit in Trypanosoma brucei.

Rink, Constance (University at Buffalo, Buffalo, NY, USA); Williams, Noreen (University at Buffalo, USA)

Trypanosoma brucei is a parasitic protozoan and causative agent of trypanosomasis. Trypanosomasis remains a significant economic and health burden in Africa. Ribosome biogenesis is a highly conserved and vital process for eukaryotes including *T. brucei*. A critical step in ribosome biogenesis is the transport of the 60S and 40S ribosomal subunits from the nucleus to the cytoplasm where they assemble into the functional ribosome. One of the nuclear export factors responsible for export of the 60S in other systems is the heterodimer TbMex67-TbMtr2. These proteins are not well characterized in *T. brucei* and have been only functionally linked to mRNA export. Our goal is to characterize the function of TbMex67-TbMtr2 in the nuclear export of the 60S, specifically, their interactions with components of the 5S RNP (P34/P37, L5 and 5S ribosomal RNA). We have recently found that TbMex67 associates with P34/P37 and L5 in wild type procyclic extracts, and RNA is not necessary in order to maintain these interactions. *In vitro*, we have shown that P34 and L5 directly interact with TbMex67. Interestingly, the addition of 5S rRNA abolishes the direct interaction between P34 and TbMex67. We examined the potential interaction between TbMtr2, P34, and L5 and found that these proteins do not directly interact. We determined TbMex67-TbMtr2 is able to bind 5S rRNA through TbMex67. Ultimately, we believe that a better understanding of the nature of the interactions between the 5S RNP and TbMex67-TbMtr2 will provide new targets for chemotherapeutic intervention.

16 Arginine methylation of TbLpn and its role in phospholipid biosynthesis in Trypanosoma brucei

Spencer, Evan (Department of Biology-The College at Brockport, USA); Pelletier, Michel (The College at Brockport, Brockport, NY, USA)

Phospholipids biosynthesis, particularly phosphatidylcholine (PC) and phosphatidylethanolamine (PE) plays a major role in the survival of *T. brucei*. Of great importance is the fact that, as opposed to other parasitic organisms, trypanosomes synthesize phospholipids *de novo*. Although the pathways for phospholipids biosynthesis have not been very well characterized, recent data have helped to better understand how trypanosomes are able to assemble phospholipids. We have identified TbLpn, a protein homologous to yeast and human lipin, a phosphatidate phosphatase involved in membrane biogenesis, energy metabolism, and adipose tissue development. We have demonstrated that, as its yeast and mammal homologs, TbLpn catalyzes dephosphorylation of phosphatidic acid (PA) to form diacylglycerol (DAG). In addition, TbLpn contains methylated arginine residues *in vivo*, and interacts with TbPRMT1, the major trypanosome Protein Arginine Methyltransferase (PRMT), suggesting that one or several arginine residues within TbLpn might be asymmetrically dimethylated by TbPRMT1.To determine whether TbLpn is indeed a TbPRMT1 substrate, and to evaluate whether any of the other four trypanosome PRMTs can methylate TbLpn, we are carrying out *in vitro* methylation assays in which bacterially expressed recombinant TbLpn (His-TbLpn) is incubated with purified recombinant TbPRMT1, TbPRMT3, TbPRMT5, TbPRMT6, or TbPRMT7, in the presence of S-adenosyl-L-methionine, followed by denaturing gel electrophoresis and western blot using antibodies specific for methylated arginine residiues. Preliminary results indicate that both TbPRMT1 and TbPRMT7 are able to methylate TbLpn *in vitro*. We are currently in the process of confirming these results using an in vitro methylation assays that utilizes S-adenosyl-L-[methyl-^{3}H]methionine.

17 Arginine methylation: a switch that modulates effector functions of DRBD18 in T. brucei

Kaur, Jan Naseer (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 14214, Buffalo, NY, USA); Lott, Kaylen (USA); Read, Laurie (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 14214., USA)

Unlike most eukaryotes, *Trypanosoma brucei* primarily relies on posttranscriptional processes to regulate gene regulation. RNA binding proteins (RBPs) play a key role in governing the fate of transcribed mRNAs at the levels of stability, translation, export, and localization. There are various examples in yeast and humans where posttranslational modifications impact the functions of RBPs. Studies in our laboratory discovered that DRBD18, an RBP conserved only in the Order Kinetoplastida, is essential for survival of both bloodstream form and procyclic form *T. brucei*. When DRBD18 is depleted, the transcriptome of *T. brucei* undergoes extensive rearrangement. We identified three methylarginines in DRBD18, and showed that the methylation status of these residues drastically alters the protein's function. Methylation of these arginine residues promotes the mRNA stabilization activity of DRBD18 and hinders its destabilization activity. The protein arginine methyltransferase, TbPRMT1, interacts with and methylates DRBD18 *in vivo*. Depletion of TbPRMT1 alters the level of putative mRNA targets in a manner similar to the effects of hypomethylated DRBD18, confirming that arginine methylation of DRBD18 acts as a switch that regulates transcriptome biology in *T. brucei*. Tandem affinity chromatography followed by mass spectrometry analysis revealed that methylmimic and hypomethylated DRBD18 associate with different ribonucleoprotein complexes, suggesting that arginine methylation significantly shapes the protein-protein interactions of DRBD18. Protein-protein interaction studies suggest that nuclear mRNA export is one of the effector pathways of DRBD18 mediated regulation. Current studies in lab support the *in vivo* interaction of DRBD18 with two of the conserved export factors; Mex67 and Mtr2. Future studies will determine the direct targets of DRBD18 and investigate how arginine methylation impacts the interactions and functionality of DRBD18.

18 Diplonemids and kinetoplastids - diverse, abundant and overlooked components of marine plankton

Lukes, Julius (Biology Center, Ceske Budejovice, CZE); Flegontova, Olga (Biology Centre, USA); Flegontov, Pavel (Biology Centre, USA); Kaur, Binnypreet (Biology Centre, USA); Faktorova, Drahomira (Biology Centre, USA); Tashyreva, Daria (Biology Centre, USA); Prokopchuk, Galina (Biology Centre, USA); Malviya, Shruti (Tata Institute of Fundamental Research, USA); Bowler, Chris (Ecole Normal Superieur, USA)

Diplonemids, a sister clade of kinetoplastid flagellates, were recently identified as the most diverse marine eukaryotes which also rank as the 6th most abundant eukaryotic group. However, only a very few species have been described and we know nothing about their life style. Marine kinetoplastids, represented mainly by free-living neobodonids, are much less abundant and diverse. To fill the large gap in our knowledge of diplonemids, we embarked on the studies of their diversity and distribution, morphology and life cycles. We are also assembling and annotating two transcriptomes and genomes that share some features with related kinetoplastid, but differ from them by f.e. abundant presence of introns. We are also trying to get representative species into the culture. Moreover, we were recently able to transform Diplonema papillatum and hope to turn it into a tractable organism suitable for functional analyses.

19 The African Trypanosome Evades Immune Clearance by Sugar-Coating its Antigenic Surface

Nesic, Dragana (Rockefeller University, USA); Pinger, Jason (Rockefeller University, USA); Ali, Liaqat (University of Dundee, USA); Kim, Hee-Sook (Rockefeller Uniersity, USA); Chowdhury, Shanin (Rockefeller University, USA); Lilic, Mirjana (Rockefeller University, USA); Cross, George (Rockefeller University, USA); Ferguson, Michael (University of Dundee, USA); Papavasiliou, Nina (German Cancer Research Center, USA); Stebbins, C Erec (German Cancer Research Center, Heidelberg, DEU)

Trypanosoma brucei subspecies cause sleeping sickness in humans and nagana in livestock, and are a leading impediment to development in sub-Saharan Africa. *T. brucei* elicits a robust antibody-mediated immune response to its exposed surface coat (**V**ariant **S**urface **G**lycoprotein, or **VSG**). The parasite counters host immunity by accessing a large genetic pool of variant VSGs to "switch" to a new, antigenically distinct protein. The 1.4Å resolution crystal structure of coat variant VSG3 manifests a heretofore unappreciated divergence in the tertiary and oligomeric structures of these surface molecules. The structure also reveals an *O*-linked carbohydrate on the uppermost surface of the VSG, a modification previously unknown in trypanosomes. Antibody binding and infection assays demonstrate that VSG3 glycysylation potently impairs immune recognition and pathogen clearance. These results establish a novel paradigm in trypanasomal infection, raising significant questions about the spectrum of VSG variability and its impact on immune evasion.

20 Photo-affinity labelling and biochemical analyses identify the target of trypanocidal bis-tetrahydropyran 1,4-triazoles as the FoF1-ATP synthase (mitochondrial complex V)

Tulloch, Lindsay B. (Univerisyt of St Andrews, St Andrews, GBR); Menzies, Stefanie K. (Univerisyt of St Andrews, USA); Fraser, Andrew L. (Univerisyt of St Andrews, USA); Gould, Eoin R. (Univerisyt of St Andrews, USA); King, Elizabeth F. (Univerisyt of St Andrews, USA); Zacharova, Marija K. (Univerisyt of St Andrews, USA); Florence, Gordon J. (Univerisyt of St Andrews, USA); Smith, Terry K. (Univerisyt of St Andrews, USA)

Current drugs to treat African sleeping sickness are inadequate and new therapies are urgently required. We previously reported the promising trypanocidal activity of compound 1, a bis-tetrahydropyran 1,4-triazole (B-THP-T) synthetic analogue of chamuvarinin. This study aims to identify the protein target(s) of compound 1 in *Trypanosoma brucei* and understand its mode of action to aid further inhibitor optimisation.

To identify targets, we functionalised compound 1 with UV-reactive diazirine and "click"-compatible alkyne substituents to generate a bi-functional photo-affinity analogue, compound 3, which retained trypanocidal activity. Bi-functional compound 3 was UV cross-linked to its target in procyclic *T. brucei in vivo* and biotin affinity or Cy5.5 fluorescent reporter tags were subsequently appended by Cu(II)-catalysed azide-alkyne cycloaddition. The biotinylated protein adducts were enriched/isolated with streptavidin affinity beads and subsequent LC-MSMS identified the FoF1-ATP synthase (mitochondrial complex V) as a potential target.

Mitochondrial complex V was confirmed to be the target via a number of biochemical assays. We show that (i) compound 1 decreases cellular ATP levels (ii) by inhibiting oxidative phosphorylation (iii) at the FoF1-ATP synthase and (iv) that our compounds interact specifically with both the α- and β-subunits of the complex. Modeling indicates that they bind within the nucleotide-binding sites at the interfaces between the α- and β-subunits. The FoF1-ATP synthase is essential to both procyclic and bloodstream forms of *T. brucei* and its identification as our target now allows us to further optimise inhibitor potency and selectivity along the drug discovery pipeline.

21 The role of polyamines for proliferation, survival, and infectivity in the protozoan parasite Leishmania donovani

Perdeh, Jasmine (Pacific University, USA); Love, Quintin (Pacific University, USA); Harrelson, John (Pacific University, USA); Boitz, Jan (Oregon Health & Sciences University, USA); Yates, Phillip (Oregon Health & Sciences University, USA); Ullman, Buddy (Oregon Health & Science University, USA); Roberts, Sigrid (Pacific University School of Pharmacy, Hillsboro, OR, USA)

A better understanding of parasite biology and host-parasite interactions is critical for the development of much needed new therapeutic strategies against the neglected tropical disease leishmaniasis, which affects an estimated 12 million people annually. Polyamines are metabolites that play central roles in the biology of all eukaryotes, and recent studies have highlighted their critical nature in *Leishmania* parasites. In *Leishmania*, the polyamine biosynthetic pathway consists of four enzymes: arginase (ARG), ornithine decarboxylase (ODC), spermidine synthase (SPD), and S-adenosylmethionine decarboxylase (ADOMETDC). These enzymes sequentially generate ornithine, putrescine and spermidine. We have generated a complete set of gene deletion mutants in *Leishmania donovani* (*LdΔarg*, *LdΔodc*, *LdΔspd*, and *LdΔadometdc*), in order to evaluate the polyamine biosynthetic enzymes as therapeutic targets. *In vitro* studies showed that the gene deletion mutants depend on the provision of downstream metabolites to survive. An evaluation of cell growth between the different cell lines uncovered that the only vital role of ornithine is as precursor for polyamine synthesis. However, putrescine, which has previously been postulated to be merely a substrate for spermidine formation, has additional essential functions. A preliminary analysis of polyamine function reveals that in *Leishmania*, putrescine may be more important for parasite proliferation, while spermidine appears to be more crucial for parasite survival. *In vivo* infectivity studies revealed that *LdΔodc* exhibits profoundly reduced infectivity compared to wild type parasites, while *LdΔspd* and *LdΔadometdc* show a less pronounced, albeit significant, reductions in infectivity. In contrast, the *LdΔarg* gene deletion has little effect on virulence. The observed discrepancies in infectivity phenotypes suggest a model in which the polyamine precursor ornithine is readily salvaged by intracellular parasites, whereas the polyamines putrescine and spermidine are severely limited.

22 Characterisation of an unusual Leishmania HSP70 family member, HRP4

Drini, Sima (Institut Pasteur, USA); Späth, Gerald (Institut Pasteur, USA); Rachidi, Najma (Institut Pasteur, Paris, FRA)

During the *Leishmania* life cycle, stage differentiations are triggered by environmental cues. Heat shock proteins (HSPs) are likely to be involved in stress sensing and thus important in regulating these processes. We identified a novel HSP, HRP4, with an unusual protein structure combining a HSP70 and a TPR domain that could play a role in differentiation. We showed that HRP4 is present in *Leishmania* and in ancestral trypanosomatids, but absent in *Trypanosoma brucei* and *T. cruzi*. We showed that HRP4 levels are high during the growth phase and decrease in stationary phase due to the degradation of HRP4 by two mechanisms: the proteasome machinery and in late stationary phase, an unknown protease. This degradation is important to regulate the morphology of parasites. In wild type (WT) parasites, the body length/width ratio (l/w) increases as cells progress from logarithmic to stationary phase to become thinner. Comparing the l/w ratio of various HRP4 mutants with the WT, we found that one mutant displayed a slender form already in logarithmic phase. Truncation of HRP4 led to early morphological changes, suggesting that its degradation in stationary phase could be important to regulate these events. As the l/w ratio increases during metacyclogenesis, HRP4 could be involved in the negative regulation of this process. HRP4 has a second function, as it acts as a heat shock protein with chaperone function. Indeed its level increases in response to high temperature, it interacts with other HSPs, HSP70 and HSP90 and it binds to ATP.

We thus identified two potential roles for HRP4, as a chaperone and as a negative regulator of the morphological changes that accompany metacyclogenesis.

23 A genome-scale screen for cell cycle defects in the African trypanosome

Marques, Catarina A. (Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK, Dundee, GBR); Glover, Lucy (Trypanosome Molecular Biology, Department of Parasites and Insect Vectors, Institut Pasteur, France, USA); Cassidy, Andrew (Tayside Centre for Genomic Analysis, School of Medicine, University of Dundee, UK, USA); Horn, David (Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK, USA)

RNA interference (RNAi) library screening in *Trypanosoma brucei* has proven to be a powerful approach for determining which proteins are, for example, required for the parasite's viability. Proteins that are essential for viability can then be considered genetically validated as potential drug-targets. Major challenges remain however, in terms of developing a deeper understanding of how these proteins impact trypanosome biology and viability. To gain further insight into protein function at a genomic scale, we have now carried out a screen for cell cycle defects. A genome-wide bloodstream form RNAi library was induced for 24 h and ~108 cells, stained with propidium iodide, were isolated by fluorescence-activated cell sorting (FACS) according to DNA content. Sub-G1, G1, S, G2/M and >G2/M populations were recovered and subjected to RNAi Target sequencing (RIT-seq). In this approach, each read serves as a barcode to report the relative representation of each gene-knockdown in each of the five isolated populations. As expected, we find that cell-cycle perturbations are enriched for gene-knockdowns previously associated with a fitness-cost. One particularly prominent feature is a >G2/M enrichment profile that emerges for cohorts of genes associated with the cytoskeleton and motility. Another notable feature is the association of proteasome components knockdowns with an accumulation at G2/M. We are currently analysing the datasets and will present findings in relation to factors involved in DNA-replication, chromosome segregation, phosphorylation and RNA-binding, among others.

24 UMSBP2 is a zinc finger protein essential for chromosome end protection in Trypanosoma brucei

Klebanov-Akopyan, Olga (Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, USA); Mishra, Amartya (Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, USA); Glousker, Galina (Department of Genetics, The Hebrew University of Jerusalem, USA); Tzfati, Yehuda (Department of Genetics, The Hebrew University of Jerusalem, USA); Shlomai, Joseph (Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, USA)

Telomeres protect the ends of eukaryotic chromosomes from degradation, recombination or detrimental fusion to other chromosome ends. They consist of short DNA repeats conserved across human and trypanosomes and bound by specialized proteins. A similar sequence, known as UMS, is conserved at the replication origin of the trypanosomal kinetoplast DNA (kDNA) minicircles and is bound by a CCHC-type zinc finger protein, the UMS binding protein (UMSBP). Here we report that one of the two UMSBP paralogs in *Trypanosoma brucei*, TbUMSBP2, colocalizes with telomeres at the nuclear periphery and is essential for maintaining their structure, protection and function. Knockdown of *TbUMSBP2* resulted in delocalization of the telomeres from the nuclear periphery, phosphorylation of histone H2A at the vicinity of the telomeres, impaired nuclear division, endoreduplication, and cell growth arrest. Furthermore, TbUMSBP2 depletion caused rapid reduction in the G-rich telomeric overhang sequence, and an increase in C-rich telomeric overhang sequence and in extrachromosomal telomeric circles. These results indicate that TbUMSBP2 is essential for the structure and function of telomeres. The sequence similarity between the mitochondrial UMS and the telomeric overhang as well as the binding of both sequences by UMSBP proteins, suggest a common function in mitochondrial DNA replication and telomere maintenance.

25 Characterization of the alkyl-dihydroxyacetonephosphate synthase TbADS of Trypanosoma brucei

Lee, Sungsu (USA); Cheung-See-Kit, Melanie (USA); Williams, Tyler (USA); Zufferey, Rachel (St John's University, Jamaica, NY, USA)

Glycerolipids account for approximately 80% of total cellular lipids and thus, are the main constituents of biological membranes in *Trypanosoma brucei*, which causes sleeping sickness in humans. They are important cellular components that fulfill various essential functions for the cell beyond their structural role in membranes, such as second messengers in signal transduction pathways, regulation of membrane trafficking, and control of cell cycle progression. While most of the glycerolipids are ester glycerolipids that bear a fatty acid at position one of the glycerol backbone, a significant proportion of glycerolipids (mainly PE and PS species) in this parasite bear an ether-linked aliphatic fatty alcohol (ether lipids) instead. We report here the biochemical characterization of the second enzyme involved in ether glycerolipid biosynthesis, the alkyl-dihydroxyacetonephosphate synthase *Tb*ADS. *Tb*ADS subcellular localization, its role in growth, viability, and glycerolipid metabolism will be presented.

26 Pentatricopeptide repeat-containing polyadenylation factor KPAF3 defines mitochondrial mRNA identity and stability in trypanosomes

Afasizheva, Inna (Boston University, Boston, MA, USA); Zhang, Liye (Boston University, USA); Sement, Francois (Boston University, USA); Suematsu, Takuma (Boston University, USA); Yu, Tian (Boston University, USA)

In *Trypanosoma brucei*, most mitochondrial mRNAs undergo internal sequence changes by U-insertion/deletion editing and 3′ end modifications. The temporally separated and functionally distinct modifications are manifested by adenylation prior to editing, and extension of short A-tails into long A/U-heteropolymers upon completion of editing. The A-tails stabilize partially and fully edited mRNAs while the A/U-tails enable mRNA binding to the ribosome. Here, we identify an essential pentatricopeptide repeat-containing (PPR) RNA binding protein, termed kinetoplast polyadenylation factor 3 (KPAF3), and demonstrate its role in protecting pre-mRNA against degradation by the mitochondrial processome. We show that KPAF3 recruits poly(A) polymerase KPAP1 to the 3′ terminus leading to mRNA stabilization or decay depending on the occurrence and extent of editing. *In vitro*, KPAF3 stimulates KPAP1 activity and inhibits RNA uridylation by RET1 TUTase. Our findings indicate that KPAF3 selectively directs pre-mRNA toward adenylation rather than uridylation, which is a default post-processing 3′ modification characteristic of ribosomal and guide RNAs. KPAF3 binding also ensures that mRNAs entering the editing pathway are adenylated and, therefore, competent for post-editing A/U-tailing and translational activation.

27 FPC5, an essential, novel and active N-kinesin required for organelle segregation and endomembrane function in Trypanosoma brucei

Perdomo, Doranda (CNRS-Université Bordeaux, USA); Berdance, Elodie (Université de Bordeaux, USA); Lallinger-Kube, Gertrud (University of Bayreuth, USA); Sahin, Annelise (Université Bordeaux, USA); Landrein, Nicolas (CNRS-Université Bordeaux, USA); Cayrel, Anne (CNRS-Université Bordeaux, USA); Ersfeld, Klaus (University of Bayreuth, USA); Kohl, Linda (CNRS-UMR7245, Muséum National d'Histoire Naturelle, USA); Robinson, Derrick R. (CNRS-Université Bordeaux, USA)

The flagellar pocket (FP) of the pathogen *Trypanosoma brucei* is an important single copy structure that is formed by the invagination of the pellicular membrane. It is the unique site of endo- and exocytosis and is required for parasite pathogenicity. The FP consists of distinct structural sub-domains with the least explored being the flagellar pocket collar (FPC). *Tb*BILBO1 is the first-described FPC protein of *Trypanosoma brucei*. It is essential for parasite survival, FP and FPC biogenesis. In this work we describe FPC5, a novel FPC protein and *Tb*BILBO1 partner. FPC5 is a putative kinesin that belongs to the exclusive Kinetoplastid group 2 of kinesins. Here we describe the functional roles of FPC5 in *T. brucei* procyclic (PCF) and bloodstream (BSF) cells. We demonstrate that purified recombinant FPC5 protein (FPC5r) is an active kinesin *in vitro*. Cellular characterization by down-regulation of FPC5 expression using RNAi induces multinucleated PCF cells with detached flagella, misplaced kinetoplasts and with the new FPC localized to the anterior region of the cell. In the case of BSF, depletion of FPC5 results in cells with a disturbed endomembrane organization, and within 48h cell arrest and death. Overall, our results characterize the first FPC protein to have kinesin activity as well as cellular functions that are associated with organelle positioning. This is the second protein to be identified at the FPC, and is also essential, indicating that the FPC is indeed a promising target for drug intervention.

28 Trypanosoma brucei RAP1 binds to telomeric DNA in a non-sequence specific manner

Gaurav, Amit K. (Cleveland State University, Cleveland, OH, USA); Li, Bibo (Cleveland State University, USA)

Trypanosoma brucei, the causative agent of human African trypanosomiasis, evades host immune response via regular switching of its major surface antigen, VSG. *T. brucei* has numerous *VSG* genes, but VSGs are expressed exclusively from expression sites located at subtelomeric regions in a monoallelic manner. Telomeres are nucleoprotein complexes located at chromosome ends and are essential for genome integrity. We have identified several *T. brucei* telomere proteins: *Tb*TRF is the duplex telomere DNA binding factor and interacts with both *Tb*RAP1 and *Tb*TIF2. We have shown that *Tb*RAP1, *Tb*TRF, and *Tb*TIF2 all suppress VSG switching, while *Tb*RAP1 is essential for subtelomeric *VSG* silencing. Genes located closer to the telomere experience stronger *Tb*RAP1-metiated telomeric silencing than genes located more upstream, indicating that localization of *Tb*RAP1 at the telomere is essential for its silencing effect. However, the means of recruiting *Tb*RAP1 to the telomere was not clear. Although the *Tb*TRF-*Tb*RAP1 interaction would presumably help target *Tb*RAP1 to the telomere, we observed WT level of telomere-associated *Tb*RAP1 in cells depleted of *Tb*TRF. Using electrophoretic mobility shift assays, we now show that the *Tb*RAP1 binds to DNA in a sequence non-specific manner and a RKRRR motif appears to be responsible for this DNA binding activity. Our data indicate the possibility of a *Tb*TRF-independent recruitment of *Tb*RAP1 to the telomere.

29 Characterising DNA-binding components of the unconventional kinetochore in Trypanosoma brucei

Nerusheva, Olga (The University of Oxford, Oxford, GBR); Treep, Joris (Department of Biochemistry, University of Oxford, USA); Marciano, Gabriele (Department of Biochemistry, University of Oxford, USA); Akiyoshi, Bungo (Department of Biochemistry, University of Oxford, USA)

The kinetochore is a multiprotein complex that localises at the centromere of eukaryotic chromosomes. During cell division, the kinetochore provides attachment of chromosomes to spindle microtubules and promotes accurate chromosome segregation. In most organisms, kinetochore assembly at the centromere depends on the centromere-specific histone H3 variant, CENP-A. However, kinetoplastids lack a homologue of CENP-A, suggesting that these species assemble kinetochores in a CENP-A-independent manner. In the past years, we identified 20 kinetochore proteins named KKT1–20 in Trypanosoma brucei, an experimentally tractable parasite, and found that they constitute kinetoplastid-specific kinetochores. To investigate the mechanism by which this unconventional kinetochore assembles at the centromere, we are characterising KKT2 and KKT3 proteins that localise at the centromere throughout the cell cycle, and therefore are likely to bind to the DNA or chromatin directly. These homologous proteins have an N-terminal kinetoplastid-specific kinase domain, central domain that contains putative DNA-binding motifs, and divergent polo-box domain at the C-terminus. Analysis of truncated forms of these proteins in vivo demonstrated that their central domains are sufficient for the centromeric localisation and that this localisation depends on several conserved residues. We also showed that the central domains can indeed bind DNA in vitro. Our RNAi analyses showed that KKT2 and KKT3 are required for the localisation of other KKT proteins. This data support the hypothesis that KKT2 and KKT3 act as essential components of the kinetoplastid kinetochore and their central domains interact directly with centromeric DNA. Using analog-sensitive allele of KKT2, we also showed that its kinase activity is indispensable for cell viability. We are currently trying to understand how KKT2-dependent phosphorylation affects the function of kinetoplastid kinetochores.

30 Trypanosoma cruzi cell cycle regulation contributes to amastigote plasticity and persistence under stress

Dumoulin, Peter C. (Harvard T.H. Chan School of Public Health, Boston, MA, USA); Burleigh, Barbara (Harvard T.H. Chan School of Public Health, USA)

Eukaryotic cells have the capability to regulate their cell cycle progression in response to mitogens and stressors. The parasite *Trypanosoma cruzi*, the causative agent of Chagas disease has the ability to replicate in the cytoplasm of a variety of mammalian hosts and tissue types. We explored the possibility that intracellular amastigotes can regulate their cell cycle in respond to a variety of stressors, allowing them to adapt and persist in dynamic host cell environments. We found that amastigotes regulate their growth primarily through a reversible G1 checkpoint in response to nutrient deprivation. This level of regulation occurs prior to each replication and allows amastigotes to adapt to suboptimal nutrient levels. Surprisingly, this response also occurs in the presence of benznidazole, the first-line therapy used against *Trypanosoma cruzi* infection. Upon exposure to benznidazole, parasites persist in the G1/0 state of their cell cycle and can subsequently divide when drug pressure is removed suggesting a predominantly static mechanism of action. Consequently, parasite plasticity through cell cycle regulation may allow for a wide host cell tropism as well as explain the inability of certain drugs to provide a sterilizing cure.

31 Ergosterol biosynthesis in Leishmania: protecting parasites in the face of danger?

Mukherjee, Sumit (Department of Biology, Texas Tech University, Lubbock, TX 79401, USA, USA); Xu, Wei (Department of Molecular Microbiology, Washington University, St. Louis, MO 63110, USA, USA); Moitra, Samrat (Department of Biology, Texas Tech University, Lubbock, TX 79401, USA, USA); Zhang, Kai (Department of Biology, Texas Tech University, Lubbock, TX 79401, USA, USA)

Unlike their mammalian hosts, *Leishmania* parasites synthesize high levels of ergosterol and other 24C-methylated sterols during the promastigote stage. While the sterol biosynthesis is down-regulated in amastigotes, its inactivation leads to attenuated virulence in mice. To reveal the roles of ergosterol synthesis during the life cycle of *Leishmania*, we characterized mutant parasites that are deficient in either sterol methyl transferase or C14-α demethylase, two key enzymes in this pathway. Despite of near normal growth under regular *in vitro* culture conditions, these mutants exhibit severe defects when challenged with stress conditions such as elevated temperature, starvation, and presence of other microbes. Given the fundamental importance of sterols to membrane property, we investigated the impact of these mutations on the functions of key cellular organelles including mitochondria and plasma membrane. So far our studies have shown that these sterol mutants have higher mitochondrial membrane potential than wild type parasites, indicative of more active mitochondria. Indeed the mitochondria in these mutants are capable of producing more ATP than those in wild type, as shown by ATP production assay. The mutants also have much higher concentrations of superoxide in the mitochondria than wild type parasites, which may cause cellular damage. Further studies are being carried out to better understand how ergosterol biosynthesis affects the cell biology of *Leishmania* and protects parasites against different types of stress.

32 The elucidation of DNA replication monitoring in trypanosomatids allows the obtainment of more accurate values for the duration of cell cycle phases

da Silva, Marcelo Santos (Butantan Institute, São Paulo, BRA); Marin Muñoz, Paula Andrea (Butantan Institute, USA); Elias, Maria Carolina (Butantan Institute, USA)

Trypanosomatids are a group of protozoan parasites that are the etiologic agents of various infectious diseases in humans. They probably diverged very early during eukaryotic evolution and have drawn attention as peculiar models for evolutionary and comparative studies. Here, we show a meticulous study comparing the incorporation and detection of the thymidine analogs BrdU and EdU in Leishmania amazonensis, Trypanosoma brucei, and Trypanosoma cruzi to monitor their DNA replication. We used BrdU- and EdU-incorporated parasites with the respective standard detection approaches: Indirect Immunofluorescence to detect BrdU after standard denaturation (2 M HCl) and "click" chemistry to detect EdU. We found an enormous discrepancy between these two thymidine analogs due to the poorly detection of BrdU, and this is reflected on the estimative of the duration of the cell cycle phases G1, S, and G2. To solve this discrepancy, we increase the exposure of incorporated BrdU using different concentrations of HCl, allowing a better BrdU detection. The results obtained showed that 3 M of HCl for 20 min is enough to making the percentage of BrdU-labeled parasites equivalent to the EdU-labeled. To confirm the accuracy of the values of the duration of cell cycle phases re-estimated, we performed another approach using EdU/BrdU 15 min pulse and quantifying the percentage of cytokinesis labeled nuclei. The results obtained supported the new most reliable values for the duration of cell cycle phases. In conclusion, we suggest that the studies using BrdU with standard detection approach, not only in trypanosomatids but also in others cell types, should be reviewed to ensure an accurate estimation of DNA replication monitoring.

33 Functional characterisation of a DYRK kinase implicated in quorum sensing in Trypanosome brucei

Cayla, Mathieu (University of Edinburgh, Edinburgh, GBR); McDonald, Lindsay (University of Edinburgh, USA); MacGregor, Paula (University of Cambridge, USA); Matthews, Keith R. (University of Edinburgh, USA)

The gene Tb927.10.15020 was identified in a genome-wide RNAi screen for molecules required for stumpy formation, selection being mediated through resistance to pCPTcAMP in vitro. This gene encodes a DYRK family kinase which are implicated in cellular quiescence and stress responses in diverse eukaryotes.

To gain mechanistic insight into the function and regulation of Tb927.10.15020, null and overexpression mutants have been constructed in *T. brucei* and recombinant protein produced in insect cells. TET-induced overexpression of the wild-type kinase in trypanosomes leads to a hyper sensitivity to pCPTcAMP, a phenotype that is not seen when an inactive mutant kinase is expressed. Using a combination of this pCPTcAMP-sensitivity phenotypic screen and in vitro kinase assays we demonstrate the essential role in kinase activity of the molecule's non-conventional DFS motif (instead of the conventional DFG motif) as well as the histidine in the HxY motif of the activation loop. Interestingly, we also found that kinase activity contributes to the regulation of Tb927.10.15020 mRNA levels. Thus, ectopic expression of the kinase in a WT or a null background does not elevate mRNA levels above those in wild-type parasites whereas mRNA levels increase 2-5 fold when the inactive kinase is expressed. In parallel, the phosphoproteome of a Tb927.10.15020 null mutant has been compared to WT cells and a set of ~20 proteins identified as being reproducibly regulated in the absence of the kinase. These molecules are currently undergoing validation with respect to their involvement in pCPTcAMP sensitivity in vitro and slender to stumpy differentiation in vivo, as well as their potential to be direct substrates of Tb927.10.15020.

34 A heterodimeric, RNA polymerase II-associated factor is required for SL RNA gene transcription, likely representing the missing transcription factor IIF in trypanosomes

Srivastava, Ankita (Department of Genetics and Genome Sciences, UConn Health, Farmington, CT, Farmington, CT, USA); Badjatia, Nitika (Department of Genetics and Genome Sciences, UConn Health, Farmington, CT, USA); Lee, Ju Huck (Department of Genetics and Genome Sciences, UConn Health, Farmington, CT, USA); Günzl, Arthur (Department of Genetics and Genome Sciences, UConn Health, Farmington, CT, USA)

Transcription initiation from a dedicated position requires the formation of a pre-initiation complex (PIC) which, for RNA pol II, consists of the general transcription factors TFIID/TBP, TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, the polymerase and, often, mediator. In trypanosomes, only SL RNA genes (*SLRNA*s), encoding the *trans*-splicing substrate, are known to form an RNA pol II PIC at their promoters. This PIC consists of extremely divergent orthologs of the SNAP complex, which replaces TFIID at small RNA gene promoters, TFIIA, TBP (TRF4), TFIIB, TFIIH/[putative]TFIIE and mediator, leaving only TFIIF to be discovered. In humans/yeast, TFIIF is a heterodimeric complex that binds directly to RNA pol II, facilitating recruitment of the enzyme to the PIC. In addition, TFIIF is crucial for the formation of a stable PIC.

We tandem affinity-purified RNA pol II of *T. brucei* and identified co-purified proteins by liquid chromatography-tandem mass spectrometry. We found all 12 RNA pol II subunits, several transcription elongation factors and proteins known to interact with the enzyme. Additionally, we consistently detected three proteins of unknown function. Two of them associated with RNA pol II in seemingly stoichiometric amounts, indicating that they form a complex. Biochemical purification and sedimentation analysis confirmed this notion. Silencing either gene rapidly interfered with cell culture growth and reduced both the relative abundance of SL RNA and the SL RNA synthesis rate. ChIP of one subunit found the protein to occupy the *SLRNA* promoter but not the *SLRNA* intergenic region or promoters of RNA pol I/III-transcribed genes. *In vitro* assays confirmed that this subunit is important for *SLRNA* transcription. Consequently, the newly identified factor may represent trypanosome TFIIF.

35 A localisation and functional study of two Leucine Rich Repeat containing proteins and their roles in cell cycle regulation in Trypanosoma brucei brucei.

Crickley, Robbie (Oxford Brookes University, Oxford, GBR); Mohr, Timm (Oxford Brookes University, USA); Vaughan, Sue (Oxford Brookes University, USA)

Here we present two paralogous leucine rich repeat containing proteins localised to the basal body – pro-basal body junction and the pro-basal body respectively using a recently developed long primer PCR only tagging method (pPOT). These proteins are of particular interest as both localise to this region in a temporally regulated manner associating their presence with specific basal body segregation and duplication events within the cell cycle of *T. brucei*.

Initial functional assays in procyclic form cells using inducible RNAi causes multiple non-canonical cell types to appear in culture ranging from zoids to multi-nucleated cells as well as poly flagellated cells and cells with irregularly high numbers of basal body pairs. Scanning electron micrographs have revealed an aberrant cell morphology at all stages of the cell cycle. A fluorescence based analysis of the overexpression cell lines has revealed the emergence of a novel fibrous structure close to the basal bodies.

These initial findings are indicative of these proteins playing a crucial role in regulating the highly controlled basal body segregation and biogenesis cycles as well as playing a critical role in ensuring correct organelle segregation. It is anticipated that further functional studies in conjunction with a search for interacting partners will further elucidate the mechanisms which underpin basal body duplication in kinetoplastidae as well as providing insights to the wider centriole biogenesis community.

36 Use of Chiral Cell Shape to Ensure Highly Directional Swimming in Trypanosomes

Wheeler, Richard J. (University of Oxford, Oxford, GBR)

Trypanosomatids undergo complex movement as they swim. Swimming of closely-related species has been considered in detail, however how large species-to-species morphological diversity confers different swimming abilities has not. Using procyclic *Trypanosoma brucei* (trypomastigote), *Leishmania mexicana* (promastigote) and *T. brucei* ClpGM6 RNAi (epimastigote-like) cells I determined how cell shape contributes to swimming behaviour. These morphologies had characteristic swimming behaviours: The promastigote followed looping swimming paths, the trypomastigote followed straight (highly directional) swimming paths and the epimastigote-like cells had slightly lower directionality paths than the trypomastigote. The geometry of these paths could be predicted from the observed rate of longitudinal rotation of the cell while swimming. A new method of high speed video analysis showed trypomastigotes had a highly chiral helical shape, distorted by the flagellar beat. Epimastigote-like cells were less chiral, and promastigotes were not chiral. The flagellum attachment zone (FAZ) appears responsible for trypomastigote chirality, as ClpGM6 RNAi shortened the FAZ and reduced chirality. Simulation showed cell shape chirality causes longitudinal rotation due to hydrodynamic drag as the cells swim, and this correlated with observed rotation. This reveals a potential function of the FAZ in modulating swimming behaviour, beyond catastrophic failure of motility in the absence of flagellum attachment. Trypomastigote cell shape may therefore be an adaptation for highly directional swimming or for certain modes of chemotaxis. Chirality of cell shape and longitudinal rotation while swimming have been recognised as common features of swimming microorganisms for over a century. This analysis of trypanosomatids showed, quantitatively, how longitudinal rotation can allow cells to avoid loss of directionality due to biological noise (morphogenetic variation and flagellar beat variation), providing a plausible general explanation for why swimming cells tend to have strong asymmetries in cell shape or propulsion.

37 Signalling pathway from glucose transporter towards FOF1-ATPase

Kovarova, Julie (University of Dundee, USA); Horn, David (University of Dundee, USA)

Bloodstream *Trypanosoma brucei* lives in its host's blood and its metabolism is reduced, adapted to such a nourishing environment. However, several studies recently found these parasites in additional tissues, such as the adipose tissue or skin. We present here a signalling pathway which would contribute to quick adaptation of metabolism when transferring from blood to a glucose depleted environment. Baker *et al*. (2015) observed that depletion of vacuolar ATPase (vATPase) enables loss of the kinetoplast, hence loss of subunit A6 of F_OF_1-ATPase, leading to F_OF_1-ATPase uncoupling. In contrast to most other organisms, the canonical function of mitochondrial ATPase (F_OF_1-ATPase) in bloodstream *T. brucei* is to sustain mitochondrial membrane potential at the expense of ATP, which is mainly produced in glycolysis. Under specific conditions (or in dyskinetoplastic trypanosomes) F_O and F_1 moieties are separated and F_OF_1-ATPase becomes uncoupled losing its function. We propose that a signalling pathway is present and dependent upon glucose availability, leading via vATPase towards F_OF_1-ATPase; depletion of glucose leads to disassembly of vATPase, which further triggers uncoupling of F_OF_1-ATPase. Inhibitors of glucose transport do indeed render the kinetoplast dispensable, as predicted by our model. We are now investigating this pathway through genetic manipulation of glucose transporters.

38 Trypanosoma cruzi cell death mechanism and physiological changes induced by an organometallic platinum compound

Mosquillo, Florencia (Facultad de Ciencias, UdelaR, USA); Smircich, Pablo (Facultad de Ciencias, UdelaR, USA); Gambino, Dinorah (Facultad de Química, UdelaR, USA); Garat, Beatriz (Facultad de Ciencias, UdelaR, USA); Perez, Leticia (Facultad de Ciencas, UdelaR, USA)

For more than 50 years, Chagas' disease treatment has been based on two broad spectrum drugs: Nifurtimox and Benznidazol. Nevertheless, the effectiveness of these drugs in chronic patients is limited and several side effects are produced due to its toxicity, being contraindicated in children and immunosuppressed patients. Therefore, the biological evaluation of new compounds with potential antichagasic activity is of great relevance. In this context, a new organometallic platinum compound, 1,1′-bis(diphenylphosphino) ferroceno pyridine-2- thiolate-1- oxide Pt(II) hexafluorophosphate (Pt-dppf-mpo), has been synthesized and characterized in previous work. Pt-dppf-mpo has shown antitrypanosomal activity in *T. cruzi* cultures with low IC_{50} values in the Dm28c strain. However, the mechanism of action underlying its antiproliferative effect against *T. cruzi* has been unexplored. In this work, we analyzed the type of death induced by the platinum compound and its effect on *T. cruzi* proliferation. Morphological changes in *T. cruzi* epimastigotes were observed after 6 and 24 hours of treatment with nanomolar concentrations of the compound. In addition, a mitochondrial dysfunction with a collapse of the mitochondrial membrane potential is induced. These events suggest that Pt-dppf-mpo incubation leads to cell necrosis with no evidence of early apoptotic-like markers. Moreover, we analyzed global changes in transcriptome and proteome of parasites incubated with the complex aiming to identify affected pathways and further characterize its mode of action. Altogether, the data here presented contribute to get a deeper knowledge of the mechanism of action of a promising antitrypanosomal compound, providing insights into the pathogenesis and helping in a rational drug design against Chagas' disease.

39 A kinetoplastid-specific alpha-helix-containing protein cooperates with CIF1 to promote cytokinesis initiation in Trypanosoma brucei

Kurasawa, Yasuhiro (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA); Zhou, Qing (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA); Li, Ziyin (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, USA)

Cytokinesis in *Trypanosoma brucei* occurs along the longitudinal axis from the anterior tip of the new flagellum attachment zone (FAZ) filament toward the posterior cell end. The regulatory pathway controlling this unusual mode of cytokinesis just starts to emerge. We recently delineated a signaling cascade composed of Polo-like kinase (TbPLK), Aurora B kinase (TbAUK1) and two kinetoplastid-specific proteins CIF1 and CIF2 that act in concert at the distal tip of the new FAZ filament to promote cytokinesis initiation (Zhou et al., 2016, PNAS; Zhou et al., 2016, JBC). Here, we report another kinetoplastid-specific protein named CIF3 that cooperates with CIF1 at the new FAZ tip to promote cytokinesis initiation. CIF3 contains three alpha-helical motifs, and through the N-terminal alpha-helix it interacts with the two zinc-finger motifs of CIF1. This *in vivo* interaction between CIF1 and CIF3 depends on TbPLK activity, indicating that they function downstream of TbPLK. Depletion of CIF3 disrupted the localization of CIF1 and TbAUK1 to the new FAZ tip, leading to inhibited cytokinesis initiation. We further demonstrated that CIF1 and CIF2 function in a complex on the new FAZ tip during S phase of the cell cycle, which is TbPLK dependent, and when CIF2 disappears from the new FAZ tip, CIF1 and CIF3 function in a complex thereafter to recruit TbAUK1 to the new FAZ tip for the latter to initiate cytokinesis. Together, these results revealed the mechanistic role of CIF3 in cytokinesis initiation, determined the order of action among these cytokinesis regulators, and delineated a cytokinesis regulatory pathway that may be shared by the kinetoplastid parasites.

40 Molecular dynamics of genome and epigenome ingetrity in Trypanosoma brucei

Kim, Hee-Sook (Rockefeller University, USA); Papavasiliou, Nina (German Cancer Research Center, USA)

In *Trypanosoma brucei*, genes are organized into polycistronic transcription units (PTUs), and transcription and replication share their initiation sites. Most of classical transcription factors are missing in *T. brucei*, but specific chromatin marks are located at transcription boundaries. These findings suggest that *T. brucei* relies on epigenetic factors to control DNA replication, transcription, and their coordination. Recently we discovered that site-specific chromatin marks are required for proper DNA replication, building a strong premise for functional interactions between chromatin structure and DNA replication. PTU boundaries are designated Transcription Start Sites (TSSs) and Transcription Termination Sites (TTSs). PTU boundaries have unique chromatin structures that consist of special histone variants, histone modifications, and a *Kinetoplastid*-specific DNA modification, base J (modified dT). In particular, TTSs are enriched with base J, H3v, and H4v, while TSSs are with base J.

We have previously shown that although H3v and base J are required for proper transcription termination, these marks are not essential for cell survival (*Schulz et al, 2016*). As H4v co-localizes with H3v and base J at TTSs, we reasoned that *H3v*Δ *J*Δ cells might be viable because H4v can compensate for the absence of H3v and J marks. We discovered that simultaneous deletion of all three marks – H3v, H4v, base J – indeed resulted in substantial cell growth defects. Interestingly, *H3v*Δ *H4v*Δ *J*Δ mutant exhibited characteristic feature of cells suffering from replication stress, including accumulation of G2 cells and sub-G1 (less than 2C DNA content) cells, and accumulation of nuclear TbRPA1 foci (an indication of abnormally exposed ssDNA lesions and replication stress). These phenotypes were highly similar to those of a mutant lacking MCM-Binding Protein (TbMCM-BP), a replication factor (*Kim et al, 2013*), priming us to assess whether DNA replication is as defective in *H3v*Δ *H4v*Δ *J*Δ as it is in *TbMCM-BP*Δ mutants. We will present these data and will discuss our working hypotheses of how site-specific epigenetic marks can control DNA replication in *T. brucei*.

41 UTRme: a tool to annotate UTRs in Trypanosomatids

Radio, Santiago (UdelaR, Facultad de Ciencias, Laboratorio de Interacciones Moleculares. Ministerio de Educación y Cultura. IIBCE, USA); Becco, Lorena (UdelaR, Facultad de Ciencias, Laboratorio de Interacciones Moleculares., USA); Sotelo, José (Ministerio de Educación y Cultura. IIBCE, USA); Garat, Beatriz (UdelaR, Facultad de Ciencias, Laboratorio de Interacciones Moleculares. , USA); Smircich, Pablo (UdelaR, Facultad de Ciencias, Laboratorio de Interacciones Moleculares., USA)

Since post-transcriptional regulation is the main level of gene expression control in trypanosomatides, the co-regulation of functionally related genes is postulated to be mainly achieved through post transcriptional operons. This system relies on signals that are mostly present in the UTRs of mRNAs, and that are recognized by regulatory trans acting factors. To deepen our understanding of the mechanisms and signals that determine these processes we need to correctly determine mRNA boundaries. To this end we developed UTRme (UTR-mini-exon), a program to annotate UTRs in trypanosomatids. Using pair-end RNA-Seq data and an annotated genome, UTRme annotates the UTRs and assigns them a reliability index, which indicates the confidence with which the polyadenylation and transplicing sites were detected. The program is based on several read filtering steps (through trimming and alignment) in order to obtain a reliable set from which the annotation will be generated. The score assigned to each site depends on the number of reads that support it, the differences between the bases of polyadenylated or transpliced read with its aligned genomic region, the number and percentage of adenines for 3'UTRs, among others. UTRme allowed us to determine the use of preferential sites according to the parasitic stage and search for signals in UTRs, both at the level of primary sequence and secondary structure, that might explain the observed co-regulation of gene family members

42 Dissecting the signaling pathway governing DNA damage-induced G2/M cell cycle checkpoint in Trypanosoma brucei

04/23/17 07:00 PM - 09:00 PM

Zhou, Qing (University of Texas Medical School at Houston, Houston, TX, USA); Li, Ziyin (University of Texas Medical School at Houston, USA)

The DNA damage-induced G2/M cell cycle checkpoint serves as a surveillance mechanism to prevent the cell from entering mitosis with genomic DNA damage, thereby maintaining genome stability and cell viability in eukaryotic organisms. This important cell cycle checkpoint, which is well understood in yeast and animals, is regulated by ATM/ATR-mediated activation of checkpoint kinases (Chks) to further inhibit the G2/M-specific cyclin-dependent kinase 1 (Cdk1) through inhibitory phosphorylation by the Wee1 kinase on Cdk1. *Trypanosoma brucei*, an early branching protozoan, appears to lack some of the key cell cycle checkpoints, such as the mitosis-cytokinesis checkpoint and the Mad2-mediated spindle assembly checkpoint. Moreover, it is also unclear whether other cell cycle checkpoints operate in *T. brucei* and how these checkpoints are regulated. Here we report a novel K homology (KH) domain-containing protein in *T. brucei*, named DDR1, which responds to DNA damage and functions as an effector in the DNA damage-induced G2/M cell cycle checkpoint. DDR1 localizes to kinetochores in around 10% of wild-type cells, but upon treatment with DNA damage agents DDR1 protein level increased ~5-fold and was detectable in almost all cells. Depletion of DDR1 by RNAi caused moderate growth defects and led to aberrant chromosome segregation. Overexpression of DDR1 arrested cells at the G2/M phase, reminiscent of DNA damage agent-treated cells in which DDR1 protein level was elevated. Finally, we also investigated the contribution of ATM/ATR in DDR1-mediated G2/M checkpoint pathway and the regulation of the G2/M-specific cyclin-dependent kinase CRK3 by DDR1 and Wee1 kinase. To our knowledge, DDR1 is the first DNA damage-induced cell cycle checkpoint regulator identified in *T. brucei*, and our results will uncover the signaling pathway governing DNA damage-induced G2/M cell cycle checkpoint in this early divergent eukaryote.

43 Chemical validation of Trypanosma brucei uridine diphosphate-N-acetylglucosamine pyrophosphorylase (UAP) as a drug target

04/23/17 07:00 PM - 09:00 PM

Major, Louise L. (University of St Andrews, St Andrews, GBR); Cockram, Peter (Univ of St Andrews, USA); Ronin, Céline (USA); Lage de Siqueira Neto, Jair (USA); Ciapetti, Paola (USA); McKerrow, James (USA); Smith, Terry (University of St Andrews, USA)

UAP is a genetically validated drug target in *T. brucei* (Stokes et al., 2008). Previously Urbaniak et al. (2013) demonstrated *in vitro* that TbUAP inhibition occurs via a kinetoplastid-specific UTP-competitive allosteric binding. This intrigued us, so we have further characterized UAP enzymes from *T. brucei*, *T. cruzi* and *L. major*.

Our biochemical characterization has included kinetic analysis and screening against all the three enzymes and crystal structures of the three enzymes including an apo *Tb*UAP structure. Compound library screens via enzyme inhibition assays and/or thermal shift assays identified a common moiety that appears to bind to the allosteric site of all three kinetoplast enzymes. However the entrance of the allosteric site is significantly different among the three enzymes.

We next synthesized a small synthetic library to explore binding and probe interactions surrounding the allosteric pocket.

In vivo inhibition of UAP activity in *T. brucei* was confirmed by multiple reactant monitoring (MRM) MS quantification of associated metabolites for three UAP fragment based inhibitors. This provides chemical validation of UAP as a drug target in *T. brucei*. Additionally, MRM-MS has identified UAP as a target for a nanomolar trypanocidal compound that was identified from a library of FDA-approved drugs. This compound is an ideal candidate for repurposing.

Stokes et al., JBC (2008) 283: 16147-16161

Urbaniak et al., ACS Chem Biol (2013) 8: 1981-1987

POSTERS: Session B
Monday 7:00 pm

04/24/17

44 Investigating the linkage between pleomorphism and antigen switch frequency

McWilliam, Kirsty (University of Edinburgh, Edinburgh, GBR); Matthews, Keith (University of Edinburgh, USA)

Laboratory-adapted lines of *Trypanosoma brucei* are reported to switch their expressed VSG at a significantly lower frequency than populations recently transmitted through tsetse flies. In the absence of tsetse transmission, these laboratory-adapted parasites also lose the capacity to differentiate into transmission-competent stumpy forms. However, it is unclear if their reduced rate of antigenic variation is linked to the loss of pleomorphism or whether the processes, although co-selected by multiple passage, are independent. Recently we used a genome-wide RNAi screen to identify regulators of stumpy formation and demonstrated that their inducible gene silencing caused parasites to remain slender *in vivo* (Mony *et al*., Nature, 2014). This provides a tool to switch pleomorphism 'on' or 'off' inducibly, without long term passage. We are exploiting this 'inducible-monomorphism' to address whether there is a concomitant change in the frequency of antigenic variation in the population.

Initially we have generated inducible RNAi lines for two components of the stumpy formation pathway using the *T. brucei* 'AnTat 90:13' cell line, that expresses VSG AnTat1.1 and allows TET-inducible gene silencing. Thereafter, we integrated a GFP reporter into the AnTat1.1 expression site (ES) at a promoter-proximal position. When the ES is active, the parasites are GFP positive as well as AnTat1.1 positive (measurable by flow cytometry). However, antigenic switches can be detected and quantitated by either loss of GFP expression (an ES switch) or loss of AnTat1.1 expression only (replacement of the active VSG in the ES). I will present the construction and validation of these cell lines and present the outcome of experiments that quantitate VSG switching in cells competent or not for stumpy formation.

45 BioID and kDNA segregation proteins in Trypanosoma brucei

Baudouin, Hélène (Institute of Cell Biology, University of Bern, Bern, CHE); Trikin, Roman (Institute of Cell Biology, University of Bern, USA); Schimanski, Bernd (Department of Chemistry and Biochemistry, University of Bern, USA); Ochsenreiter, Torsten (Institute of Cell Biology, University of Bern, USA)

Almost all eukaryotes require proper distribution of mitochondria and their genomes during cell division. However, this process is poorly understood in all major model organisms. I use *Trypanosoma brucei* to study this problem. *T. brucei* only contains one mitochondrion with one genome, the kinetoplast (kDNA). It has been suggested that the kDNA is connected to the basal body of the flagellum *via* the Tripartite Attachment Complex (TAC), which is essential for proper kDNA segregation. But only few components are known. Our lab identified a key component, TAC102, the only known mitochondrial matrix protein of the TAC. Since we know that TAC102 does not directly interact with the kDNA, other TAC components must exist that connect the genome to TAC102.

I used proximity labelling of TAC102 to identify new TAC proteins. For this, TAC102 was fused with a promiscuous biotin ligase (BirA*) that biotinylates in close proximity all proteins, which were then enriched and identified by mass spectrometry.

Myc-BirA*-TAC102 behaves chemically the same as the endogenous TAC102, and immunofluorescence analysis showed a colocalization of TAC102 and Myc signals. However, continuous overexpression of Myc-BirA*-TAC102 leaded to slower growth of parasites.

To start BioID analysis, cells were incubated with biotin, and Myc-BirA*-TAC102 expressed. The biotinylated proteins were enriched, and identified by mass spectrometry. A condition where Myc-BirA*-TAC102 was not expressed served as a negative control.

Reassuringly, the most enriched protein was TAC102. Most of the identified proteins are predicted as mitochondrial. I analyzed four biological replicates and created a list of candidates based on their enrichment during Myc-BirA*-TAC102 expression. I will present data on the candidates and their potential function in the TAC.

46 Genome-wide analysis of mitochondrial translation of Trypanosoma brucei using Ribosome Profiling

Mesitov, Mikhail (Department of Molecular and Cell Biology, Boston University School of Dental Medicine, USA); Yu, Tian (Department of Molecular and Cell Biology, Boston University School of Dental Medicine, USA); Afasizheva, Inna (Department of Molecular and Cell Biology, Boston University School of Dental Medicine, USA)

Most mitochondrial mRNAs of T. brucei are encrypted and need to be edited by insertion/deletion of uridine residues to create open reading frames. Once editing is completed, long 200 - 250 nt A/U tail is added byKPAP1 poly(A) polymerase and RET1 TUTase in a reaction that also requires KPAF1/KPAF2 polyadenylation factors. The A/U-tail activates translation by increasing mRNA affinity for the small ribosomal subunit. However, the mechanisms that control translation initiation on short 5′ untranslated regions (UTRs), editing status of translated mRNAs, and translational kinetics remain unknown. Because highly hydrophobic nature of mitochondrial proteins limits proteomics approaches to assess the composition and relative abundance of mitochondrially-encoded proteome, we adapted ribosome profiling to investigate these aspects of mitochondrial gene expression. Our approach is based on isolating translating ribosomes by density gradient centrifugation and rapid affinity purification followed by deep sequencing of ribosome protected fragments (RPFs). Our analysis revealed uneven ribosome occupancy of pan-edited mRNAs, such as RPS12 and A6, with characteristic peaks on 5′ and 3′ UTRs. Conversely, most unedited mRNAs typified by CO1, ND4 and ND5 appear to be uniformly translated. Our results demonstrate applicability of ribosome profiling to investigate translational control in mitochondria of T. brucei.

47 Inorganic polyphosphate interacts with nucleolar and glycosomal proteins in trypanosomatids

S. Negreiros, Raquel (University of Georgia, USA); Lander, Noelia (University of Georgia, USA); Smith, Stephanie (University of Illinois at Urbana-Champaign, USA); H. Morrissey, James (University of Illinois at Urbana-Champaign, USA); Huang, Guozhong (University of Georgia, USA); Docampo, Roberto (University of Georgia, USA)

Inorganic polyphosphate (polyP) is a polymer of three to hundreds of phosphate units bound by high-energy phosphoanhydride bonds and present from bacteria to humans. Most polyP in trypanosomatids is concentrated in acidocalcisomes, acidic calcium stores that possess a number of pumps, exchangers, and channels, and are important for their survival. In this work, using polyP as bait we identified > 50 putative protein targets in cell lysates of *Trypanosoma cruzi* and *T. brucei*. Gene Ontology analysis of the binding partners found a significant overrepresentation of nucleolar and glycosomal proteins. Interestingly, using the polyphosphate-binding domain (PPDB) of *Escherichia coli* exopolyphosphatase we localized long chain polyP to the nucleolus and glycosomes of trypanosomes. A competitive assay based on the pre-incubation of PPBD with exogenous polyP and subsequent immunofluorescence assay of procyclic forms of *T. brucei* showed polyP dose-dependent and chain length-dependent decrease in the fluorescence signal. Targeting of yeast exopolyphosphatase to the glycosomes of procyclic forms resulted in alteration in their glycolytic flux and increase in the susceptibility to oxidative stress.

48 RAB11 in the secretory pathway of Trypanosoma brucei

Umaer, Khan (USA); Bush, Peter (USA); Bangs, James (USA)

Rabs are small GTPases that regulate vesicular trafficking in the secretory and endocytic pathways. *Trypanosoma brucei* possesses a streamlined secretory system that guarantees efficient delivery to the cell surface of the critical virulence factor, GPI-anchored variable surface glycoprotein (VSG). VSG is constantly endocytosed and recycled to the flagellar pocket via the TbRab11-positive recycling endosome. In addition, receptor-mediated endocytosis of host transferrin is essential for the parasite growth, and it has been assumed that recycling of the GPI-anchored transferrin receptor (TfR) occurs via the same route. An inducible bloodstream form RNAi cell line was created, and established markers were used to query the effect of TbRab11 depletion on specific pathways. TbRab11 is essential for bloodstream trypanosomes, but ablation does not affect turnover or steady state levels of TfR. Nor is biosynthetic delivery of VSG to the cell surface affected. A newly developed method to assay surface protein (VSG) recycling confirms no effect of knockdown on bulk recycling. These results suggest that TbRab11 does not play function in *de novo* surface trafficking or recycling of GPI-anchored cargo. However, TbRab11 depletion dramatically increases lysosomal turnover of the trans-membrane surface protein ISG65, suggesting a role in recycling of non-GPI anchored proteins. TbRab11 depletion also causes cells to shed more VSG into the media by formation of extracellular vesicles as judged by scanning electron microscopy. Unexpectedly, TbRab11 silencing reduced receptor-mediated endocytosis, a phenomenon we ascribe to secondary effects. Together, our results indicate a role of TbRab11 in the homeostatic maintenance of the secretory/endocytic system of bloodstream *T. brucei*.

49 The canonical poly(A) polymerase PAP1 polyadenylates non-coding RNAs and is essential for snoRNA biogenesis and the role of snoRNAs in rRNA processing in Trypanosoma brucei

Chikne, Vaibhav (Bar-Ilan university, USA); Kumar Gupta, Sachin (Bar-Ilan University, USA); Doniger, Tirza (Bar-Ilan University, USA); Michaeli, Shulamit (Bar-Ilan University, Ramat-Gan, ISR); Cohen Chalamish, Smadar (Bar Ilan University, USA); Rajan, K. Shanmugha (Bar-Ilan University, USA); Waldman Ben-Asher1, Hiba (Bar Ilan university, USA); Unger, Ron (Bar-Ilan university, USA); Ullu, Elisabetta (Yale University, USA); Kolev, Nikolay G. (Yale University, USA); Tschudi, Christian (Yale University, USA); Michaeli, Shulamit (Bar-Ilan University, USA)

Trypanosomes possess two canonical RNA poly (A) polymerases termed PAP1 and PAP2. PAP1 is encoded by one of the only two genes harboring a *cis*-spliced intros in this organism and its function is currently unknown. Here, we show that PAP1, which is located in the nucleus, polyadenylates non-coding RNAs, which undergo *trans*-splicing and polyadenylation. Upon RNAi-induced silencing of either PAP1 or PAP2 the level of snoRNAs is reduced. The dual polyadenylation of snoRNA intermediates is mediated first by PAP2 and then by PAP1 and requires the factors essential for polyadenylation of mRNA. As opposed to most eukaryotes, the trypanosome large subunit (LSU) rRNA is fragmented to two large and four small subunits (srRNA). This additional processing requires trypanosome-specific functions (proteins and RNA). Thus, in trypanosomes, snoRNAs are involved in guiding modification on RNAs, but also in rRNA processing. Here we demonstrate the role of eight abundant snoRNAs in the processing of the LSU rRNA. Purification of Ssf1p an LSU processing factor with its associated proteins identified 104 rRNA processing factors. The results suggest that the special fragmentation of rRNA in trypanosomes is mediated by trypanosome-specific snoRNAs but also requires most of the factors involved in rRNA in other eukaryotes.

50 Conserved Motifs in Nuclear Genes Encoding Predicted Mitochondrial Proteins in Trypanosoma cruzi

Becco, Lorena (Laboratorio de interacciones moleculares, Facultad de ciencias, Universidad de la República, USA); Chavez, Santiago (Laboratorio de interacciones moleculares, Facultad de ciencias, Universidad de la República, USA); Smircich, Pablo (Laboratorio de interacciones moleculares, Facultad de ciencias, Universidad de la República, USA); Duhagon, María Ana (Laboratorio de interacciones moleculares, Facultad de ciencias, Universidad de la República, USA); Garat, Beatriz (Laboratorio de interacciones moleculares, Facultad de ciencias, Universidad de la República, USA)

Trypanosoma cruzi, the protozoan parasite that causes Chagas' disease, exhibits peculiar features that make it a good model to study post-transcriptional regulation. The presence of a unique mitochondrion in the kinetoplastid protozoans is remarkable. In spite of the fact that the mitochondrial DNA constitutes up to 25% of total cellular DNA, the structure and functionality of the mitochondrion are absolutely dependent on the expression of the nuclear genome. As in other eukaryotes, the mitochondrial localization of nuclear encoded proteins in trypanosomatids is achieved through specific peptide signals. However, there are mitochondrial proteins encoded in the nuclear genome that lack of a peptide signal. Alternative, protein targeting to subcellular organelles via mRNA localization has been also recognized and specific mRNA localization towards the mitochondria has been described in other eukaryotes. Seeking for mitochondrial localization signals in *T. cruzi*, we built a database of nuclear genes encoding predicted mitochondrial proteins, which was named MiNT. A conserved peptide signal, M(L/F/R)(R/S/T/A)(R/S)SSRR, named TryM-TaPe was found in 61% of the database. In addition, the search for compositional signals in the transcripts of MiNT enabled the identification of a conserved element, RDAARRD, amongst others. Since the latter motif is recognized by the *T. brucei* TRRM3 protein, which is enriched in mitochondrial membrane fractions, a putative zipcode role is suggested for this element. Globally, we provide an inventory of the mitochondrial component proteins in *T. cruzi*, and found out conserved peptide and nucleotide motifs that may constitute mitochondrial localization signals.

51 Assessing the presence of functional trypanosome lytic factors in human breast milk

Ebenezer, Karen (Hunter College, Brooklyn, NY, USA); Verdi, Joey (Hunter College, USA); Raper, Jayne (Hunter College, USA)

Breast milk is the primary source of nutrition for newborns, containing a range of nutrients, and innate immune factors that protect infants from infection. Human milk hosts immune factors, such as active complement proteins and maternally transferred IgA_2 antibodies, but also has significant lipid character. High-density lipoprotein (HDL) complexes circulate in plasma, and transport cholesterol and lipid throughout the human body. These complexes are characterized by the presence of the structural cholesterol acceptor protein apolipoprotein A-1 (APOA1). Recently human breast milk proteome analyses have indicated the presence of APOA1. However, the relative amount of HDL in breast milk remains unclear and its presence has not been validated by conventional biochemistry. Additionally, our innate immune system is fortified by a specific subset of high-density lipoproteins, called trypanosome lytic factors (TLFs), named for their ability to kill an infective unicellular parasite called the African trypanosome. These TLFs contain two unique proteins in addition to APOAI: Haptoglobin-related protein (HPR) and apolipoprotein L-1 (APOL1), the latter of which induces the cellular death of a trypanosome through pore formation. Till now, APOL1 has not been detected in any human breast milk proteome analyses, despite the known presence of APOA1. Using various purification procedures and biochemical assays, we investigated whether TLF complexes were present in human breast milk, whether it possessed a detectable degree of functional APOL1, and whether the presence of these innate immune factors can confer potential physiological benefits to neonatal immune systems.

52 Elucidating the role of membrane contact sites in calcium signaling pathway of Trypanosoma brucei.

RAMAKRISHNAN, SRINIVASAN (Center for Tropical and Emerging Global Diseases, Athens, GA, USA); Huang, Guozhong (USA); Docampo, Roberto (USA)

Membrane contact sites are regions of close apposition between two organelles typically less than 30 nanometers apart and facilitate transfer of biomolecules. Presence of such contact sites has been demonstrated in yeast, plant and mammalian cells. However, their existence and role in protozoan parasites remains to be investigated. Here we investigated the presence of such contact sites in the protozoan parasite, *Trypanosoma brucei*. In mammalian cells, ER-mitochondria contact sites facilitate Ca^{2+} uptake by mitochondria. However, acidocalcisomes are the major Ca^{2+} storage site in trypanosomes and they possess an inositol 1,4,5-trisphosphate receptor for Ca^{2+} release. Therefore we hypothesized that in trypanosomes, acidocalcisome-mitochondria contact sites may be involved in Ca^{2+} transfer to the mitochondria. Using super-resolution microscopy we have determined that membrane contact sites exist between the acidocalcisomes and mitochondria of *Trypanosoma brucei*. Further we have confirmed the close association of these organelles using electron microscopy and proximity ligation assays. To further test Ca^{2+} transfer through these contact sites, we are currently engineering parasite lines expressing genetically encoded Ca^{2+} indicators in mitochondria. Next, we will test if the *T. brucei* mitochondrial Ca^{2+} uniporter (MCU) accumulates near these acidocalcisome-mitochondria contact sites. Lastly, we plan to identify inter-organellar tethers that may facilitate the close association and regulation of acidocalcisome-mitochondria membrane contact sites. Ca^{2+} transfer to the mitochondria is crucial for regulating the activity of mitochondrial enzymes and autophagy. Therefore, characterization of these contact sites may be a necessary starting step towards unraveling the role of Ca^{2+} in regulating the overall parasite bioenergetics.

53 Lipid rafts and dynamic palmitoylation in protozoan parasite Trypanosoma brucei

Sharma, Aabha (Northwestern University, Chicago, IL, USA)

Trypanosoma brucei, *Trypanosoma cruzi* and *Leishmania sp* employ different strategies for surviving within the mammalian host. While several proteins have been identified as virulence factors in each organism, little is known about how these factors may be regulated, for example, by post translational modifications. One such post translational modification common to all eukaryotes is S-palmitoylation, the reversible addition of palmitic acid to cysteine residue of a protein by a palmitoyl acyl transferase (PAT) enzyme. Dynamic palmitoylation then occurs by removal of this moiety by an acyl protein thioesterase (APT) enzyme. The reversible nature of this modification allows palmitoylated proteins to be shuttled on and off the membrane, thereby regulating membrane dependent function of APT substrates. We characterized the single APT in *T. brucei*, which is most similar to the human APT2 identified in a lipid raft proteome of *T. brucei* procylic parasites. Indeed the biochemical properties of TbAPT and human APT 2 are similar. Both knockdown and overexpression of TbAPT resulted in decreased parasite virulence in mice, despite there being no notable *in vitro* phenotype. Since TbAPT is a single acyl-thioesterase in an organism with 12 PATs, it is likely that a defect in proper protein palmitoylation homeostasis mediates the virulence defect and that improper regulation of specific protein palmitoylation is responsible. We are currently identifying substrates of TbAPT to further understand dynamic palmitoylation in *T. brucei*.

54 A Quorum Sensing-independent Path to Stumpy Development in Trypanosoma brucei

Zimmermann, Henriette (USA); Subota, Ines (USA); Kramer, Susanne (USA); Janzen, Christian (USA); Jones, Nicola (USA); Engstler, Markus (University of Wuerzburg, Wuerzburg, DEU)

African trypanosomes escape the mammalian host's immune system by antigenic variation of their variant surface glycoprotein (VSG) coat. VSGs are expressed from a specialized region in the genome, the expression site (ES), that contains essential expression site associated genes (ESAGs). So far, it was assumed that only "successful" antigenic switches to an intact expression site are viable. Here we suggest that "unsuccessful" VSG switches are not a dead-end, but may rather contribute to the persistence of the trypanosomes on the population level. We have simulated an unsuccessful VSG switch in pleomorphic trypanosomes by expression of a second VSG from a locus without ESAGs. In contrast to monomorphic cell lines, the pleomorphic parasites responded with surprising phenotypic plasticity. All parasites immediately exchanged the surface coat and reduced the transcripts of the ES. However, depending on the degree of ES-attenuation, the transgenic trypanosomes either resumed growth (with a new ectopic VSG coat), or stopped proliferation. We show that the growth-arrested populations rapidly and synchronously differentiate to the stumpy life cycle stage and readily passage through the tsetse fly. This occurs at very low cell densities and in the absence of the quorum sensing factor SIF. We further suggest that this not only happens in response to ectopic VSG overexpression, but also in wild-type slender populations and with rather high frequency. Thus, unsuccessful VSG switches are not lethal and cell density-dependent quorum sensing is not the only path to the tsetse fly.

55 Potential new roles for the histone methyltransferase DOT1B in Trypanosoma brucei ?

Eisenhuth, Nicole (Department of Cell and Developmental Biology, Würzburg, Germany, USA); Butter, Falk (Institute of Molecular Biology (IMB), Mainz, Germany, USA); Janzen, Christian J (Department of Cell and Developmental Biology, Würzburg, Germany, USA)

DOT1 enzymes are evolutionarily-conserved histone H3 lysine 79 (H3K79) methyltransferases, which can be found in most eukaryotic organisms. H3K79 methylation is associated with many cellular processes such as telomeric silencing, transcriptional control, cell cycle regulation, DNA repair, and developmental differentiation.

Trypanosoma brucei has two DOT1 orthologous, DOT1A and DOT1B, which are responsible for methylation of H3K76, the equivalent of H3K79 in other organisms. DOT1A catalyses mono- and di-methylation of H3K76, which is crucial for initiation of DNA replication. Tri-methylation of H3K76 is carried out by DOT1B, and is essential for developmental differentiation of bloodstream form parasites to insect-stage forms. Interestingly, the impaired differentiation in DOT1B-depleted cells is accompanied by karyokinesis defects and accumulation of DNA damage. DOT1B is also involved in other chromatin-related mechanisms such as derepression of silent telomeric expression sites (BES) in bloodstream forms, kinetics of BES switching, and BES silencing during differentiation. However the molecular machineries, which enable DOT1B to execute these different functions, are still uncharacterised.

In other organisms, DOT1 enzymes carry out their tasks as part of multi-protein complexes. The identification of these complexes provided valuable information about regulation and function of H3K79 methylation. We therefore employed a biochemical approach to purify DOT1B-containing protein complexes in *T. brucei*. Using a tandem affinity purification-based protocol, we identified several DOT1B-interacting proteins, which surprisingly suggest roles for DOT1B in replication, transcriptional regulation, and maintenance of genome integrity.

56 Direct interaction of TbMORN1 with membrane phospholipids

Sajko, Sara (Max F. Perutz Laboratories, USA); Zimmerman, Henriette (Universität Würzburg, USA); Setiawan, Kim (Universität Würzburg, USA); Djinovic-Carugo, Kristina (Max F. Perutz Laboratories, USA); Morriswood, Brooke (University of Würzburg, Würzburg, DEU)

Membrane occupation and recognition nexus (MORN) repeats are 23-amino acid motifs that are predicted to have a beta-hairpin structure. They have been implicated in protein-lipid interactions, although direct evidence for this function is lacking. In *Trypanosoma brucei*, which has 18 MORN-repeat proteins, the best-characterised is TbMORN1. TbMORN1 is present in a hook-shaped complex that coils around the neck of the flagellar pocket above the flagellar pocket collar. It is thus in close apposition to the cytoplasmic leaflet of the plasma membrane and well-positioned to interact with lipids. Recombinant TbMORN1 interacted with several phospholipid species in dot blot assays, and fluorescence anisotropy assays showed that it bound the endocytic effector phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) with micromolar affinity. Use of truncation constructs indicated that the binding site is present in the C-terminal part of TbMORN1, and mutagenesis experiments were carried out in vitro and in vivo. Previous work on TbMORN1 has shown that RNAi-mediated depletion in bloodstream form cells results in a BigEye phenotype, indicative of a possible endocytosis defect. Endocytosis is thought to be upregulated in the non-proliferative stumpy form of *T. brucei*, and TbMORN1 expression was found to be upregulated in this life cycle stage, again suggesting a link to endocytic traffic.

57 Cytosolic iron-sulfur assembly pathway interacts with cytosolic ribonucleoprotein granules in Trypanosoma brucei

Pena-Diaz, Priscila (Czech Academy of Sciences, Ceske Budejovice, CZE); Haindrich, Alexander C. (USA); Basu, Somsuvro (USA); Pierik, Antonio J. (USA); Lill, Roland (USA); Lukeš, Julius (USA)

Cytosolic Fe-S cluster proteins are involved in a variety of essential cellular processes and their assembly (CIA) pathway is conserved in all eukaryotes. These proteins process the final transfer of the Fe-S cluster to apoproteins. The CIA targeting complexes proved essential for the procyclic and bloodstream forms of *Trypanosoma brucei* and showed a novel regulatory role on mitochondrial and cytosolic aconitase. A cryo-entrapment supported pull-down of the CIA components identified three distinct complexes formed by TbCia1-TbCia2B, TbCia1-TbMms19-TbCia2B and TbCia1-TbCia2A, and revealed the mutually exclusive binding of TbCia1 to TbCia2A or TbCia2B. Localized to cytosol, the CIA complex proteins were found interacting with a number of proteins involved in ribonucleoprotein granules, namely nuclear periphery granules and p-bodies. *In-situ* tagging of RNA helicase VASA a known component of nuclear periphery granules, confirmed co-localization with the CIA components TbCia1, TbCia2B and TbCia2A. Similar results were obtained with processing bodies (p-bodies) component, SCD6. Further supporting relationship between the CIA pathway and ribonucleoprotein granules, sinefungin-mediated granule formation also recruited members of the CIA pathway. Combined, the data provide insight into the structure of this CIA module in *T. brucei* and identify their novel regulatory features.

58 Suramin Resistance is associated with a VSG switch in Trypanosoma brucei

Wiedemar, Natalie (Swiss Tropical and Public Health Institute, Basel, CHE); Wenzler, Tanja (Swiss Tropical and Public Health Institute, USA); Schmidt, Remo S. (Swiss Tropical and Public Health Institute, USA); Kunz Renggli, Christina (Swiss Tropical and Public Health Institute, USA); Cal, Monica (Swiss Tropical and Public Health Institute, USA); Ndomba, Emiliana (Swiss Tropical and Public Health Institute, USA); Graf, Fabrice E. (Swiss Tropical and Public Health Institute, USA); Mäser, Pascal (Swiss Tropical and Public Health Institute, USA)

Under a high *in vitro* suramin pressure, *Trypanosoma brucei* is able to recover within days and grow new cultures with massively lower suramin susceptibility. This selection is highly reproducible even with clones. To investigate the mechanism leading to resistance, mRNA sequencing of one *T. b. rhodesiense* clone and four independently selected derivatives of that clone was performed. Gene expression analysis revealed very few differentially expressed transcripts, all of them encoding *variant surface glycoproteins* (*VSG*). De-novo assembly of the reads mapping to those transcripts showed a switch to the same *VSG*, termed VSG^{sur}, in all the four selected derivatives. Interestingly, when looking at other clones and another strain selected with suramin, they as well showed a switch to VSG^{sur}. To investigate a possible contribution of *expression-site associated genes* (*ESAG*) in the resistance phenotype, and to differentiate between gene conversion and expression-site switch, de-novo assembly of *ESAG7* was performed. Three of the resistant derivatives showed expression of the identical *ESAG7* as the sensitive parent clone, whereas the fourth had five single-nucleotide polymorphisms within the coding sequence of *ESAG7* when compared to the sensitive parent clone. Thus, the majority of the resistant lines probably had switched their *VSG* through gene conversion. To further analyse the involvement of VSG^{sur} in the resistance phenotype, the originally expressed *VSG* is reintroduced into the active expression-site of a resistant line replacing VSG^{sur}. Characterization of the transgenic parasites will help to quantify the contribution of VSG^{sur} to the observed *in vitro* suramin resistance.

59 Target identification for a new series of natural product inspired inhibitors in bloodstream Trypanosoma brucei

King, Elizabeth (University of St. Andrews, St. Andrews, Fife, GBR); Gould, Eoin (University of St Andrews, USA); Fraser, Andrew (University of St Andrews, USA); Menzies, Stefanie (University of St Andrews, USA); Zacharova, Marija (University of St Andrews, USA); Tulloch, Lindsay (University of St Andrews, USA); Florence, Gordon (University of St Andrews, USA); Smith, Terry (University of St Andrews, USA)

Nature is a largely untapped, rich source of biologically active compounds that have been utilised to treat a wide range of diseases such as cancer and bacterial infections. Despite the use of crude plant extract in traditional medicine, natural products are rarely used to treat parasitic diseases such as Human African Trypanosomiasis, Chagas disease and Leishmaniasis. Chamuvarinin, a natural product found in the roots of the bush banana plant *Uvaria chamae*, has low micromolar activity against the mammalian bloodstream form of *Trypanosoma brucei*. Inspired by chamuvarinin, simplified analogues were synthesised and the structure activity relationship was probed. One analogue, EG96, with comparable inhibitory activity to the natural product was chosen to identify possible protein targets in the parasite. To accomplish this, analogues were synthesised with a handle for attachment of affinity and fluorescent tags and the ability to photo-crosslink. Following treatment of these compounds in the parasite, purification and mass spectrometric analysis resulted in the identification of calmodulin and centrin-4 as potential binding partners in *T. brucei* bloodstream form. To validate these as protein targets of EG96, RNA interference of the genes in the parasite has highlighted calmodulin as the likely target of interest. Validation using recombinant protein is underway and will shed more light on the mode of action directing the design of more potent and selective inhibitors. The work presented will encompass a spectrum of techniques from compound synthesis to biochemical analysis and genetic manipulation of parasites.

60 TbPH1: a kinetoplastid-specific pleckstrin homology domain containing kinesin-like protein

Kaltenbrunner, Sabine (Biology Center, Czech Academy of Sciences, Institute of Parasitology, Ceské Budejovice, CZE); Hashimi, Hassan (Institute of Parasitology Biology Center, Czech Academy of Sciences, USA); Lukeš, Julius (Institute of Parasitology, Biology Centre, ASCR, Czech Republic, USA)

The kinesin and kinesin-like protein superfamily is among one of the largest in *Trypanosoma brucei*, with almost 100 encoding genes dispersed throughout the genome. Among these is a gene encoding a protein we call *TbPH1* (Tb927.3.2490), which contains a pleckstrin homology (PH) domain that inspired its name. The 110 kDa multidomain protein is made up of an N-terminal kinesin domain whose Walker A motif is ablated by a single substitution, an intervening coiled-coil region, followed by PH and helix-turn-helix domains. While its role as a microtubule motor is suspect, it bears other motifs that suggest interactions with other proteins, lipids and even double-stranded nucleic acids. RNAi-silencing of TbPH1 in procyclic (PCF) and long slender bloodstream (BSF) forms compromises parasite fitness, likely due to a cell cycle defect resulting in an accumulation of 1N2K cells. *In situ* N- and C-terminal epitope-tagging reveals an interesting, somewhat punctate localization pattern that is distributed throughout the cell but often enriched between the nucleus and kinetoplast. Its localization predominantly excludes the lumen of such organelles as the mitochondrion, endoplasmic reticulum and acidocalcisomes. Fractionation of *T. brucei* into cytoskeleton and soluble fractions does not support TbPH1 being a component of the former. However, TbPH1 is trapped by a technique called microtubule sieving which uses the cage-like microtubule corset of the parasite as a molecular sieve. This sieve lets cytosolic proteins leave the cell while keeping larger elements like stress-granules inside. The protein is then released when the corset is depolymerized in high salt conditions. This result suggests that either TbPH1 interacts to some capacity with microtubules or is localized in some sort of granule or yet uncharacterized organelle. The trypanosome ortholog of kinesin family member 11 (TbKIF11; Tb927.9.15470) co-immunoprecipitates with TbPH1 under high salt conditions. In opisthokont model systems, KIF11 has been shown to play a key role in bipolar spindle assembly and elongation during mitosis as a homotetramer. The observation that TbPH1 may interact with TbKIF11 hints at the possibility that they may work in concert in an organism that undergoes closed mitosis.

61 Exploiting CRISPR-Cas9 technology to investigate individual histone modifications

Vásquez Ospina, Juan José (Research Center for Infectious Diseases, University of Würzburg, Josef-Schneider-Straße 2/ D15, 97080 Würzburg, Germany, USA); Wedel, Carolin (Research Center for Infectious Diseases, University of Würzburg, Josef-Schneider-Straße 2/ D15, 97080 Würzburg, Germany, USA); Cosentino, Raúl O. (Research Center for Infectious Diseases, University of Würzburg, Josef-Schneider-Straße 2/ D15, 97080 Würzburg, Germany, USA); Siegel, T. Nicolai (Research Center for Infectious Diseases, University of Würzburg, Josef-Schneider-Straße 2/ D15, 97080 Würzburg, Germany, USA)

Histone modifications can modulate DNA accessibility and are therefore a key regulator of all DNA-templated processes, including transcription and replication.

However, barring few exceptions, mutational analyses of histones *in vivo* are lacking and for most histone modifications it is not known whether mutation of a modified histone residue reproduces the phenotype caused by mutation of the modifying enzyme. Reasons for the lack of such mutational analyses are that histones are encoded by multigene families and that tools to simultaneously edit multiple genomic loci at high efficiency are being only recently developed.

To overcome these challenges and to study the function of individual histone modifications we took advantage of the recently uncovered potential of the RNA-guided endonuclease Cas9 for precise genome editing and the fact that in *Trypanosoma brucei* most DNA repair occurs via homologous recombination. DNA repair by homologous recombination allows for more precise editing than repair by non-homologous end joining.

We established an episome-based CRISPR-Cas9 system for *T. brucei* that allowed us to edit wild type cells without the need to insert selectable markers. Using our approach we were able to insert a GFP tag between the SCD6 ORF and its 3´UTR, delete both alleles of a gene in a single transfection, and perform precise editing (histone H4K4 to H4R4) of genes existing in multi-copy arrays with negligible off-target effects.

We expect the newly established genome editing toolbox to be highly useful for the trypanosome community and beyond as it allows for the generation of precise mutants without the need to change other regions of the genome, opening up opportunities to study the role of individual histone modifications.

62 How do DNA breaks contribute to mosaic VSG formation in Trypanosoma brucei?

Romero-Meza, Gabriela (Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA); Leitao, Joshua (Johns Hopkins Bloomberg School of Public Health, USA); Mugnier, Monica (Johns Hopkins Bloomberg School of Public Health, USA)

Unraveling the mysteries of antigenic variation in *Trypanosoma brucei* has been a challenge for many years. *T. brucei* uses a set of Variant Surface Glycoprotein (VSG) encoding genes to outsmart the mammalian immune system by regularly switching VSG expression in a monoallelic fashion. Studies have revealed that of the ~2,000 VSG genes in the genome, 80% are incomplete genes or pseudogenes. These genes can be used in gene conversion events to form mosaic VSGs, but the mechanisms driving this process are poorly understood. Previous experiments have shown that the induction of an exogenous DNA double strand break (DSB) near the actively expressed VSG can induce VSG switching, but the role of DSBs in the formation of mosaic VSGs has not been deeply explored. The CRISPR/Cas9 system is an ideal molecular tool for inducing targeted DSBs in any desired genomic locus, including both active and silent VSGs. Using this system, we can generate DSBs in a number of sites, allowing us to investigate the requirements for gene switching and mosaic formation. Using anchored multiplex PCR, a targeted sequencing approach that is capable of detecting gene fusions, insertions, deletions and point mutations, we will not only quantitatively monitor the formation of mosaic VSGs after the induction of a DSB, but also identify the donor sequences used to assemble a new variant. To complement *in vitro* studies, we will use this approach to monitor mosaic formation *in vivo*. This research could provide important insights into the mechanisms of VSG diversification in *T. brucei*.

63 Histone acetylation and its associated proteins are major players in defining transcription initiation sites in Trypanosoma brucei

Kraus, Amelie J. (Research Center for Infectious Diseases (ZINF), University of Wuerzburg, Würzburg, DEU); Vanselow, Jens T. (Rudolf Virchow Center for Experimental Biomedicine, University of Wuerzburg, USA); ElBashir, Rasha (Rudolf Virchow Center for Experimental Biomedicine, University of Wuerzburg, USA); Wedel, Carolin (Research Center for Infectious Diseases (ZINF), University of Wuerzburg, USA); Janzen, Christian J. (Department of Cell and Developmental Biology (Zoology I), University of Wuerzburg, USA); Förstner, Konrad U. (Research Center for Infectious Diseases (ZINF), University of Wuerzburg, USA); Schlosser, Andreas (Rudolf Virchow Center for Experimental Biomedicine, University of Wuerzburg, USA); Siegel, T. Nicolai (Research Center for Infectious Diseases (ZINF), University of Wuerzburg, USA)

In many eukaryotes, including *Trypanosoma brucei*, nucleosomes at transcription start sites (TSSs) contain H2A.Z, an evolutionary highly conserved variant of histone H2A. Many studies have addressed the H2A.Z deposition process and its role in transcription regulation. Incorporation of H2A.Z is thought to destabilize the chromatin structure and to increase DNA accessibility, important for transcription initiation. Complex patterns of histone variant distribution and a large number of TSSs have complicated further studies in higher eukaryotes. Since *T. brucei* contains only a relatively small number of chromatin factors, we analyzed the H2A.Z deposition pathway in this unicellular, eukaryotic parasite.

Using quantitative mass spectrometry, we have identified the acetylation patterns of TSS-histones and the histone acetyltransferases (HATs) responsible for these modifications. Our results suggest that the two essential enzymes, HAT1 and HAT2, mediate TSS-acetylation in different ways and that HAT2-mediated TSS-acetylation is essential for H2A.Z deposition. In addition, we have found H2A.Z deposition to be dependent on bromodomain-containing factors (BDFs). BDFs are known to bind to acetylated lysines and may recruit chromatin remodeling complexes to TSSs.

Taken together, the accurate quantification of histone acetylation allowed us to determine the function of HAT1 and HAT2 at TSSs and their role in H2A.Z deposition. Additionally, we have identified a new role for trypanosomal BDFs as key components in H2A.Z incorporation at TSSs. Our findings indicate that the H2A.Z deposition pathway in trypanosomes is similar to the pathway described for other eukaryotes supporting the idea of an evolutionary conserved mechanism of histone variant deposition.

64 RNA Editing TUTase 1: structural foundation of substrate recognition, complex interactions and drug targeting

Suematsu, Takuma (Boston University, Boston, MA, USA); Rajappa-Titu, Lional (University of Geneva, USA); Munoz-Tello, Paola (University of Geneva, USA); Long, Marius (University of Geneva, USA); Demir, Özlem (University of California, San Diego, USA); Cheng, Kevin J (University of California, San Diego, USA); Stagno, Jason R. (University of California, Irvine, USA); Luecke, Hartmut (University of California, Irvine, USA); Amaro, Rommie E (University of California, San Diego, USA); Afasizheva, Inna (Boston University, USA); Thore, Stéphane (University of Geneva, USA); Afasizhev, Ruslan (Boston University, USA)

Terminal uridyltransferases (TUTases) execute 3' RNA uridylation across protist, fungi, metazoan and plant species. Uridylation plays a particularly prominent role in RNA processing pathways of kinetoplastid. In their mitochondria, most mRNAs are internally modified by U-insertion/deletion editing while guide RNAs (gRNAs) and rRNAs are U-tailed. The founding member of TUTase family, RNA editing TUTase 1 (RET1), is not only required for 3′ uridylation on mature gRNAs, rRNAs and some mRNAs, but also participates in nucleolytic processing of precursors transcribed from maxicircle and minicircle genomes. As a subunit of mitochondrial 3 processome (MPsome), RET1 functions in uridylation of gRNA precursors prior to their processing by DSS1 3'-5 exonuclease, and in secondary uridylation of mature gRNAs. Apart from gRNA processing, RET1 also participates in mRNA translational activation by contributing Us to 200-300 nt-long A/U heteropolymers at the 3' termini. RET1 is divergent from human TUTases and is essential for parasite viability in the mammalian host and the insect vector. Given its robust *in vitro* activity, RET1 represents an attractive target for trypanocide development. Here, we report high-resolution crystal structures of the RET1 catalytic core alone and in complex with UTP analogs. These structures reveal a tight docking of the conserved nucleotidyl transferase bi-domain module composed of Pol-β DNA polymerase-like catalytic metal binding and UTP-binding domains, with a RET1-specific C2H2 zinc finger and RNA recognition (RRM) domains. Furthermore, we define RET1's region required for incorporation into the MPsome, determinants for RNA binding, subunit oligomerization and processive UTP incorporation by the mutational and biochemical analyses.

65 The translationally controlled tumor protein (TCTP) in T. brucei

Jojic, Borka (University of Bern, Bern, CHE); Amodeo, Simona (Insitute of Cell Biology, USA); Ochsenreiter, Torsten (Inistute of Cell Biology, USA)

TCTP is a highly conserved protein expressed in all eukaryotes. Several studies have reported its involvement in growth and development, cell cycle, protection against cellular stresses and apoptosis, indicating for a multifunctional role of the protein. Here, for the first time, we studied the expression and function of TCTP in the protist parasite *T. brucei*, a distantly related organism to the commonly studied Metazoans. Contrary to previous reports describing only one TCTP gene, we found two TCTP paralog genes present in *T. brucei* and named them TbTCTP1 and TbTCTP2. Both genes have an identical 5'UTR and 10 nucleotide changes in the open reading frame that lead to 5 changes at the amino acid level. The 3'UTRs of TbTCTP1 and TbTCTP2 differ in base composition and length. We show that the mRNAs of the paralogues are differentially expressed during *T. brucei* life cycle: TCTP1 is almost exclusively expressed in procyclic forms (PF) whereas TCTP2 in bloodstream forms (BSF). We found that the differential expression is attributed to the different 3'UTRs of the mRNAs. TCTP appears to be localized in the cytosol and does not change localization during the cell cycle. Silencing of TCTP by RNAi in PF results in growth retardation accompanied by an accumulation of cells exhibiting a tadpole-like morphology. Moreover, downregulation of TCTP lead to a decrease in number but increase in size of acidocalcisomes and appearance of accumulations within the mitochondrial network. This is the first characterization of the highly conserved protein TCTP in the Excavates. It provides insights in to the potential function of TCTP and characterizes the mechanism of the differential regulation of the two paralogs in *T. brucei*.

66 Investigating the function of Translesion DNA Polymerase Nu (PolN) in Trypanosoma brucei

Zurita-Leal, Andrea (University of Glasgow, USA); Lemgruber, Leandro (University of Glasgow, USA); McCulloch, Richard (University of Glasgow, USA)

Many DNA repair pathways have been documented in *Trypanosoma brucei* but less attention has been paid to damage tolerance, a reaction in which lesion bypass is needed, in particular to ensure continued genome replication. Such bypass is promoted by translesion DNA polymerases (TLS Pols). *T. brucei* has ~15 TLS candidate genes, only two of which have been functionally examined to date. Understanding the roles provided by TLS Pols could reveal new aspects of *T. brucei* biology. Here, we describe the activity of TLS Pol Nu (PolN) in bloodstream cells. Epitope tagging demonstrates that PolN localizes in discrete sites at the periphery of the nucleus in the absence of damage, with a more widespread, but non-uniform localization after damage. RNAi against PolN results in slowed growth after ~24 hours, which is associated with altered DNA content, changed cell morphology and sensitivity to DNA damage. Surprisingly, growth and morphology defects are reduced after ~48 hours, without apparent RNAi reversion. EdU labelling and γH2A analysis after PolN knockdown reveals a decrease in proliferating cells, which accumulate nuclear DNA damage. Finally, we show that PolN interacts with a nuclear putative non-canonical PolyA polymerase. Taken together, these data suggest PolN may promote *T. brucei* nuclear DNA replication.

67 The targeting complex of the cytoplasmic FeS cluster assembly machinery is essential in Trypanosoma brucei

Tonini, Maiko (University of St Andrews, St Andrews, GBR); Smith, Terry (University of St Andrews, USA); MacNeill, Stuart (University of St Andrews, USA)

Iron-Sulphur clusters (FeS) are ubiquitous protein cofactors present in all domains of life and are involved in a plethora of cellular processes. Dozens of dedicated proteins in compartmentalised machineries (cytosolic, mitochondrial, apicoplastical) tightly regulate both the assembly and transfer of FeS into target apoproteins. In eukaryotic cells, nuclear and cytosolic target proteins receive FeS from the targeting complex of the Cytoplasmic Iron Sulphur Cluster Assembly Machinery (CIA). In the cytosol, such target proteins have roles in intracellular iron homeostasis and amino acid metabolism, while in the nucleus they are implicated in DNA damage repair and replication. Although the knowledge on the CIA machinery has expanded in the past few years (Basu *et al*. 2014, Mol Microbiol., 93 [5]: 897-910), our understanding of this pathway in trypanosomatids, especially its late acting part, called CIA targeting complex still lags behind that of other eukaryotes. Here we provide an insight into the essentiality of members of this complex in the procyclic (PCF) and bloodstream (BSF) stages of the parasite via RNAi knockdown and their involvement with the transfer of FeS into target proteins as well as regulation of intracellular iron levels.

Most notably, knockdown of TbMip18 resulted in an impaired growth phenotype in both life stages of *T. brucei*, although this was more pronounced in the PCF stage. Furthermore, knockdown of TbMip18 in PCF parasites resulted in an increased number of cells retained in the G1 phase of the cell cycle with concomitant impairment in the activity of cytosolic FeS proteins. Correspondingly, lower levels of protein-bound iron in the cytosol as well as an increased sensitivity to the iron chelator deferoxamine were also observed in these cells, suggesting the involvement of the CIA targeting complex and FeS proteins in the regulation of intracellular iron levels. Surprisingly, TbMip18 knockdown cells were not more susceptible to DNA damage caused by various agents, despite the fact that many DNA damage repair proteins contain FeS and are conserved in *T. brucei*. Also, the ectopic overexpression of another member of the CIA targeting complex, TbMms19, did not interfere with the cell growth, although a dominant negative growth phenotype was observed when an ectopic copy of a truncated C-terminal domain of TbMms19 was overexpressed, which was also accompanied by partial G1 arrest. However, overexpression of the C-terminal domain did not interfere with the activity of target FeS proteins in the cytosol or sensitivity against deferoxamine.

Finally, using a combination of Tandem Affinity Purification/Mass Spectrometry and immunoblots, we evaluated the interactome of this

proteinaceous complex, encompassing not only members of the CIA pathway but also transient interactions with target FeS proteins in *T. brucei*.

Altogether, this study furthers our understanding of the FeS metabolism in *T. brucei* and highlights its contribution to a variety of cellular processes.

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68 Characterization of heterochromatin formation in Trypanosoma brucei brucei

Carloni, Roberta (University of Edinburgh, Edinburgh, GBR); Staneva, Desislava (Edinburgh University, USA); Matthews, Keith (Edinburgh University, USA); Allshire, Robin (Edinburgh University, USA)

Our aim is to investigate the mechanism for heterochromatin formation in *T. brucei brucei*, the ancient eukaryotic single-cell parasite that causes African Sleeping Sickness. Cytological analyses demonstrate the presence of distinctive heterochromatin around the periphery of trypanosome nuclei, yet its composition remains unknown.

Nucleosomes, the fundamental unit of chromatin, are formed by DNA and core histones, which are highly conserved proteins across most eukaryotes. Distinct post-translational modifications (PTMs) of histones are associated with active euchromatin or repressive heterochromatin, which influence other processes such as DNA repair. The organization of genomes into actively expressed euchromatic and inactive heterochromatic regions, and their regulation, is important for responding to endogenous and exogenous stimuli and adapting to different environmental conditions.

In most eukaryotes heterochromatin formation is mediated by methylation of histone H3 on lysine 9 and chromo-domain proteins that bind this modification. However, *T. brucei* histones are divergent and lack H3K9; this suggests that heterochromatin is formed by a distinct mechanism in these parasites. Determining how trypanosome heterochromatin is formed should provide insight into gene silencing mechanisms which may be important for host immune-evasion mechanisms and parasite development.

To begin to explore the details of trypanosome heterochromatin formation, our approach has been to GFP *in situ* tag all putative readers, writers and erasers of histone modifications in the trypanosome in order to establish their localization. Proteins identified as being heterochromatin-associated will be used to identify DNA sequences, histone modifications and other factors involved in heterochromatin formation in the different developmental forms of *T. brucei*.

69 R loops: DNA:RNA hybrids with critical roles in the genome biology of Trypanosoma brucei

Briggs, Emma (University of Glasgow, USA); Lemgruber, Leandro (University of Glasgow, USA); Crouch, Kathryn (University of Glasgow, USA); McCulloch, Richard (University of Glasgow, USA)

During transcription, nascent RNA can bind to the template strand of unwound DNA, forming stable R loops. Several proteins prevent and remove R loops, including ribonuclease H (RNase H), which digests hybridised RNA. Although threatening to genome stability, evolutionarily conserved R loop-forming sequences have been documented as powerful regulators of DNA replication, gene expression and recombination. As a kinetoplastid, *T. brucei* co-transcribes its nuclear genes from a small number (~200) of polycistronic transcription units (PTU), with adjacent units separated by poorly characterised promoters and terminators. Using genome-wide RNA:DNA hybrid mapping by so-called DRIP-seq, we have localised R loops to intergenic regions throughout the PTUs, with signal enrichment proportional to the length of the intergenic region. Conversely, we find scarce R-loop enrichment in the (sub)telomeric loci containing Variant Surface Glycoprotein (VSG) genes. *T. brucei* encodes three RNaseH proteins. RNAi targeting of RNase H2 causes cessation of *T. brucei* growth and spreading of RNA:DNA signal into the ORF of genes. Conversely, RNase H1 is non-essential and its loss in null mutants has little impact in the PTUs, but causes increased R loop signal at centromeres and across subtelomeric VSG arrays. Additionally, loss of either RNase H1 or H2 results in increased RNA:DNA signal across the telomeric VSG expression sites, including in the 70bp-repeats, with evidence for loss of VSG silencing, although this appears to occur via different mechanisms. Overall we reveal distinct and overlapping roles for two RNase H enzymes in *T. brucei*, with RNase H2-regulated R loops contributing to gene expression and RNA:DNA hybrids targeted by both enzymes acting in antigenic variation.

70 Characterisation of three independent T. brucei 'double-expressor' strains showing simultaneous activation of two VSG expression sites

Kerry, Louise (Imperial College, USA); Davies, Carys (Imperial College, USA); Budzak, James (Imperial College, USA); Pegg, Elaine (Imperial College, USA); Witmer, Kathrin (Imperial College, USA); Hall, Belinda (Imperial College, USA); Kushwaha, Manish (Imperial College, USA); D'Archivio, Simon (University of Nottingham, USA); Wickstead, Bill (University of Nottingham, USA); Rudenko, Gloria (Imperial College, USA)

Antigenic variation in *Trypanosoma brucei* is mediated by switching between expression of different Variant Surface Glycoprotein (VSG) genes located in telomeric VSG expression sites (ES). Only one VSG is expressed at a time from 1 of 15 ESs. Little is known about how monoallelic exclusion of VSG is controlled, despite its importance for parasite survival. We inserted constructs with drug resistance genes into two different ESs. Using drug selection, we selected for cells which showed simultaneous activation of both ESs. We generated three 'double-expressing' (DE) *T. brucei* lines with the VSG221 ES (marked with eGFP) and the VSGV02 ES (marked with mCherry) both simultaneously active. Generation of DE lines is rare ($<10^8$) and their stability without selection is variable. Interestingly, when drug selection pressure for the two active ESs is removed, DE lines revert back to either the VSG221 or VSGV02 ESs and not to another silent ES. This indicates there may be "memory" for a previously active ES. Flow cytometry quantitating expression of mCherry or eGFP has allowed us to characterise changing dynamics of ES expression in these independently generated DE lines. These *T. brucei* DE lines show different degrees of stability of the DE phenotype, which is reflected in differences revealed through RNA-seq analysis. This indicates that different sets of events occur to maintain a DE state. However some genes are universally up or downregulated in all three DE lines. These include the telomere binding protein TRF, which has earlier been shown to be key for ES control. We are analysing hits from our RNA-seq data to determine if perturbing their expression levels affects ES control.

71 Genome-wide mutagenesis and multi-drug resistance in Trypanosoma cruzi induced by benznidazole

Campos, Monica (LSHTM, USA); Phelan, Jody (LSHTM, USA); Francisco, Amanda (LSHTM, USA); Taylor, Martin (LSHTM, USA); Lewis, Michael (LSHTM, USA); Pain, Arnab (KAUST, USA); Clark, Taane (LSHTM, USA); Kelly, John (LSHTM, USA)

Benznidazole is the front-line drug used against *Trypanosoma cruzi* infections. However, treatment failures are widely reported. Benznidazole is a pro-drug and is bioactivated by the parasite nitroreductase TcNTR-1, leading to the generation of reactive metabolites which have trypanocidal activity. To better assess drug action and resistance, we sequenced the genomes of *T. cruzi* Y strain (35.5 Mb) and three benznidazole-resistant clones derived from a single drug-selected population. This revealed genome-wide mutagenesis in the resistant parasites, and copy number variations. In the three clones analysed, we identified a total of the 26,495 point mutations, 8,523 in coding regions, with half resulting in alterations to protein sequence. We observed mutations in a range of DNA repair genes, linked with increased susceptibility to DNA alkylating and inter-strand cross-linking agents. Stop-codon-generating mutations in*TcNTR-1* were associated with cross-resistance to several nitroheterocyclic compounds. Unexpectedly, we found that the clones were also highly resistant to posaconazole (6-22 fold), an unrelated drug proposed for use against *T. cruzi* infections, in combination with benznidazole. We therefore identify the highly mutagenic activity of benznidazole metabolites in *T. cruzi*, demonstrate that this can give rise to multi-drug resistance, and indicate that vigilance will be required if benznidazole is to be used in combination therapy.

72 The damage signalling kinase ATR plays a role in Trypanosoma brucei genome maintenance and antigenic variation

Stortz, Jennifer Ann (University of Glasgow, Glasgow, GBR); Lemgruber-Soares, Leandro (University of Glasgow, USA); Mottram, Jeremy C (University of York, USA); McCulloch, Richard (University of Glasgow, USA)

To avoid clearance by the mammalian immune system, *Trypanosoma brucei* parasites stochastically switch their surface expressed variant surface glycoprotein (VSG), selecting a new coat gene from an archive of approximately 2000 VSG variants. Each cell expresses a single VSG from a subtelomeric expression site (ES) at any given time; a process known as monoallelic expression. To date, several activities have been demonstrated to act in maintaining monoallalic expression, including chromatin status, telomere maintenance, nuclear membrane structure and potentially kinetoplastid-specific factors. DNA repair activities have mainly been considered to act in VSG switching by recombination, not monoallelic expression. Kinetoplastids encode at least two protein kinases (PKs) that use phosphorylation to mediate the response to DNA damage, but neither has been examined in detail. Here, we show that RNAi-mediated loss of ATR, a PK central to DNA repair in other eukaryotes, compromises cell proliferation, de-regulates the cell cycle and leads to widespread nuclear and chromosomal damage. In addition, ATR RNAi results in the loss of monoallelic expression, as seen by increased transcripts from the silent VSG expression sites and the appearance of cells expressing two VSGs on their surface. These data suggest a link between nuclear damage surveillance and monoallelic VSG expression.

73 High frequency of RNA leishmania-virus associated with clinical samples of mucosal leishmaniasis caused by L. braziliensis but not L. panamensis

Echeverry, Maria (Universidad Nacional de Colombia, Bogota, COL); Parra-Muñoz, Diana Marcela (Universidad Nacional, USA); Ovalle-Bracho, Clemencia (Centro Dermatologico Federico Lleras-Bogota-Colombia, USA)

Leishmania (*Viannia*) parasites are predominant in South America where they generate complicated forms of the diseases such as mucosal leishmaniasis (ML) and non-responding cutaneous leishmaniasis (NRCL).

The occurrence of the above-mentioned complications has been associated with the presence of a cytoplasmic virus in the infecting parasite, known as Leishmania RNA virus (LRV-1). However, the frequency of the virus in clinical samples ranges from 0 to 87%. The apparent inconsistent results could obey to: the use of a diverse sort of experimental approaches, an overlook of the parasite species and the type of clinical samples being analysed.

In order to avoid detection bias when looking for LRV-1 in clinical samples, the present study used complementary approaches to ensure the reliability in the LRV-1 informed frequencies.

44 Samples were analysed from different clinical backgrounds: 18 samples from ML patients and 26 from NRCL patients.

When results data were discriminated according to LRV-1 detection experimental approach, the observed frequencies were: RT-PCR: 15%, RT-qPCR: 25%, Nested PCR: 72.7%. When presented according to: clinical condition: ML: 61%, NRCL: 40%; by type of sample: biopsies: 72.7% and isolates: 45.5%; parasite species: *L. brazilinsis*: 100% and *L. panamensis*: 0%.

No difference in infectivity to macrophages or in Glucantime® susceptibility was observed between *L. braziliensis*-LRV-1^{+} and *L. braziliensis*-LRV-1^{-}.

Findings from the present study indicate that LRV-1 detection is highly dependent on the type of sample, the parasite load and the experimental approach. Also, *L. braziliensis*-LRV-1^{+} frecuency is higher *in* ML than in NRCL. There is a higher detection of the virus in biopsies than in isolates and this was found in *L. braziliensis* but not in *L. panamensis.74*

74 Intrinsic DNA curvature in trypanosomes

Smircich, Pablo (Laboratorio de Interacciones Moleculares, Facultad de Ciencias, UdelaR. Montevideo, Uruguay., USA); El-Sayed, Najib M. (Department of Cell Biology and Molecular Genetics and Center for Bioinformatics and Computational Biology, University of Maryland. MD, USA., USA); Garat, Beatriz (Laboratorio de Interacciones Moleculares, Facultad de Ciencias, UdelaR., USA)

The secondary structure of DNA has long been recognized as a signal in biological processes and more recently, its involvement in transcription initiation in *Leishmania* was proposed. In order to study whether this feature is conserved in trypanosomatids, we undertook a genome wide search for intrinsic DNA curvature in *Trypanosoma cruzi* and *Trypanosoma brucei*. Using a region integrated intrinsic curvature (RIIC) scoring that we previously developed, a non-random distribution of sequence-dependent curvature was observed. High RIIC scores were found to be significantly correlated with transcription start sites in *T. cruzi*, which have been mapped in divergent switch regions, whereas in *T. brucei*, the high RIIC scores correlated with sites that have been involved not only in RNA polymerase II initiation but also in termination. In addition, we observed regions with high RIIC score presenting in-phase tracts of Adenines, in the subtelomers of the *T. brucei* chromosomes. As these regions harbor the variable surface glycoproteins (VSG) genes this result might point towards a link between high intrinsic curvature and the recombination process that takes place at these chromosomal locations. Overall, these findings reinforce the relevance of indirect DNA readout in these ancient eukaryotes.

75 Application of a T. brucei whole-genome gain-of-function library toward identification of genes that promote drug resistance: methodological development and preliminary findings

Quinn, Mckenzie (The George Washington University, Washington, DC, USA); Gomez, Stephanie (The George Washington University, USA); Schulz, Danae (Harvey Mudd College, USA); Hovel-Miner, Galadriel (The George Washington University, USA)

Treatment of African trypanosomiasis in humans and livestock relies on a small number of effective drug treatments. Drug resistance is a persistent threat against the containment of this potentially devastating parasitic infection. Previously, the application of a whole-genome knock-down library was used to identify genes and pathways associated with anti-Trypanosomal drug resistance. This has accelerated our understanding of drug efficacy and proteins that could be involved in resistance. Here we applied a recently completed *Trypanosoma brucei* whole-genome gain-of-function library to perform complementary genetic screens to identify genes and pathways able to promote drug resistance. To date, we have finalized our methods and applied them to screening for genes that promote resistance to existing treatments against African trypanosomiasis.

76 Completion of a Trypanosoma brucei whole-genome gain-of-function library

Gomez, Stephanie (The George Washington University, Washington, DC, USA); Schulz, Danae (Harvey Mudd College, USA); Kim, Hee-sook (The Rockefeller University, USA); Papavasiliou, Nina (The Rockefeller University, USA); Hovel-Miner, Galadriel (The George Washington University, USA)

African trypanosomes are tsetse vector transmitted unicellular parasites that result in devastating human and livestock infections throughout sub-Saharan Africa. In addition, *Trypanosoma brucei* has demonstrated its usefulness as a model organism toward elucidating fundamental principles in diverse research areas that include cell biology, molecular genetics, and epigenetics. In spite of decades of active discovery in all areas of trypanosome research, more than 60% of the T. brucei genome is annotated as hypothetical genes of unknown function. Further progress in understanding both the pathogenesis and basic biology of Trypanosome species requires the development of versatile approaches for genome-scale functional analysis. Strides have been made using an available RNAi-based whole-genome loss-of-function library, yet a complementary gain-of-function library had not been produced. While individual gene studies have proven the usefulness of overexpression studies, whole-genome overexpression approaches had been hindered by the complexities involved in trypanosome gene expression. Using up-to-date *T. brucei* genome annotations and ribosomal profiling data we aimed to generate a PCR amplicon-based overexpression library. Initially PCR products were pooled based on ORF size and cloned into a standard Gateway cloning vector (pENTR) and a *T. brucei*-specific destination vector (pDEST). Following assessment of library coverage that resulted from the original 22 size-sorted pools, we observed that a significant number of ORFs were not represented in the Gateway libraries. The missing ORFs were then isolated from the original PCR plates (using robotics designed for cherry picking) and sorted into an additional 8 size-sorted pools for cloning. The quality of both sets of plasmid libraries has been fully assessed using Illumina sequencing technology and the targeted genes are well represented in final plasmid libraries. Following their testing and validation, the plasmid and resulting parasite-cell based libraries will be made broadly available to Trypanosome researchers with the fundamental goal of advancing scientific discoveries in this field.

77 Chemical genetics to study the function of deubiquitinases in Leishmania differentiation.

Damianou, Andreas (University of Glasgow, USA); Mottram, Jeremy (University of York, USA); Rodenko, Boris (UbiQ, USA); Burchmore, Richard (University of Glasgow, USA); Celie, Patrick (Netherlands Cancer Institute, USA)

Deubiquitinating enzymes (DUBs) are a class of peptidases whose function is to cleave the post-translational modifier ubiquitin from proteins or ubiquitin-conjugates. DUBs play crucial roles in many biological processes such as protein degradation, gene regulation, epigenetics, trafficking, and DNA repair. Leishmania has a ubiquitin system and its genome suggest the presence of 20 DUB orthologues, however, the identity, function and essentiality of DUBs in Leishmania remains to be revealed. A chemical proteomics approach using a fluorescent ubiquitin-based probe was used for activity-based protein profiling, revealing the presence of many active DUBs in *Leishmania mexicana*. A number of stage-specific DUBs have been identified, including some that are active during differentation of procyclic promastigote to amastigote and some that have amastigote-specific activity. Genetic evaluation using conventional knockout approaches as well as the diCRE inducible gene deletion system (Duncan *et al*.,2016) were applied to assess the essentiality of DUBs and to investigate their function. Preliminary data suggesting that at least 2 DUBs are essential. Furthermore, CRISPR-Cas9 genome editing system has been applied to endogenously tag all the predicted DUBs to investigate their localisation duringthe cell cycle and life cycle of the parasite. Finally, an active recombinant LmDUB1 protein has been expressed and purified using a baculovirus expression system and an HTS-compatible fluorescence polarisation assay developed based on the proteolysis of tetramethylrhodamine-labelled Lys(Ub)Gly. Our approach combines chemical and genetic strategies to identify essential Leishmania DUBs and to determine their function.

78 Protein networks link base J to RNAPII-mediated transcription

Jensen, Bryan C. (Center for Infectious Disease Research, Seattle, WA, USA); Gillespie, Mark (Institute for Systems Biology, USA); Phan, Isabelle (Center for Infectious Disease Research, USA); Heidebrecht, Tatjana (Department of Biochemistry, Netherlands Cancer Institute, USA); Perrakis, Anastassis (Department of Biochemistry, Netherlands Cancer Institute, USA); Ranish, Jeffrey (Institute for Systems Biology, USA); Myler, Peter (Center for Infectious Disease Research, USA)

One of the distinguishing features of kinetoplastid protozoa is glucosylation of ~1% of thymidine residues to yield β-D-Glucopyranosyloxymethyluracil (base J). J biosynthesis is a two-step process involving hydroxylation of thymidine by one of two enzymes (JBP1 or JBP2) and further modification of the resultant hydroxyl group by a glucosyltransferase (HmdUGT). JBP1 appears to be involved in maintenance of J modifying thymidines in regions already containing J, which are recognized by its J-binding domain. In contrast, JBP2 does not bind J, but has SWI2/SNF2 domain (usually associated with chromatin modification) and appears to be involved in *de novo* synthesis of J in regions lacking existing J. Little is known about how HmdUGT is recruited to the genomic locations where its enzymatic activity is needed. To identify additional proteins involved in J biosythesis, we tandem affinity purification (TAP)-tagged JBP1, JBP2 and HmdUGT, expressed them in *Leishmania tarentolae*, and identified co-purified proteins by quantitative mass spectrometry (MS/MS). JBP1 did not appear to have significant interactions with any other protein; while a single protein (J2IP1) was significantly enriched in JBP2 TAP purification. Reciprocal TAP-purification of J2IP1 confirmed the interaction with JBP2. J2IP1 contains a TUDOR domain, suggesting that it may recognize dimethylated arginine residues on chromatin-associated proteins (such as histones) to recruit JBP2 to specific genomic locations with the appropriate epigenetic markers.

Eight proteins co-purified with HmdUGT, of which five were components of the prefoldin complex. The others were a protein phosphatase (PP1C) and two hypothetical proteins (GTIP1 and GTIP2). GTIP1 contains mostly WD40 repeats, while molecular modeling of GTIP2 suggests that it contains a J-binding domain that has subsequently been comfirmed experimentaly (we have renamed it JBP3). All three proteins were TAP-tagged and the resultant complexes affinity purified and analyzed by MS/MS. These experiments indicated that HmdUGT, PP1C, GTIP1 and GTIP2/JBP3 (and another protein with homology to the mammalian PNUTS/PPP1R10) form a complex similar to the PTW/PP1 complex in other eukaryotes; suggesting that GTIP1 is the homologue of WDR82 (which also contains WD40 repeats), and JBP3 is the functional equivalent of TOX4 (a chromatin-binding protein). JBP3 also interacts with four proteins that are likely to chromatin-modifying activity

(including a histone-lysine methyltransferase), as well as two proteins associated with transcription. One of the latter (LEO1) is part of the PAF1 complex, which is involved in RNA polymerase II-mediated transcription elongation. This information now allows us to propose a novel molecular model for the role of base J in pausing/termination of RNAPII transcription.

79 Compiling a minicircle genome for Trypanosoma brucei

Yu, Tian (Boston University, USA); Zhuang, Liye (Boston University, USA); Monti, Stefano (Boston University, USA); Afasizhev, Ruslan (Boston University, USA)

Trypanosoma brucei is a protozoan parasite causing public health issues in many developing countries, whereas it also harbors a highly complex mitochondrial genome composed of maxicircles and minicircles. Expression of most maxicircle genes in *T. brucei* requires extensive mRNA sequence modification by U-insertion/deletion editing, which is guided by guide RNAs (gRNAs). These small non-coding RNAs are transcribed from approximately 10,000 minicircles. We applied focused sonication of purified kinetoplast networks to isolate full-length 1 kb minicircles for direct sequencing on PacBio Single Molecule Real-time (PacbioSMRT) platform. By clustering full-length minicircle reads we complied a near complete profile of *T. brucei* minicircle genome. We identified 240 unique minicircles, including 51 sequences previously recorded in public databases. By sequencing small RNAs from purified mitochondrial fraction, we mapped guide RNA genes and revealed a positive correlation between minicircle copy numbers and gRNA abundance. By analyzing the gRNAs and anti-sense gRNA-like molecules, we discovered a conserved sequences which likely function as promoter regions for gRNA transcription. To enable public access to our data, we constructed an annotated online database for the minicircle genome featuring gRNA locations and other conserved domains.

80 Processing and targeting of Cathepsin L (TbCatL) to the Lysosome in Trypanosoma brucei

Koeller, Carolina M. (University at Buffalo, Buffalo, NY, USA); Bangs, James D. (University at Buffalo, USA)

Pathogenic African trypanosomes (*Trypanosoma brucei ssp*.) depend on essential lysosomal activities that are differently regulated through its life cycle. Cathepsin L (TbCatL), a cysteine protease, is important to this process. TbCatL is synthesized as a mixture of two precursor forms (full length P and smaller X; X identity is uncertain) with an N-terminal prodomain that mediates post-Golgi sorting, and which is removed upon arrival in the lysosome. To examine TbCatL trafficking we developed an RNAi cell line targeting the non-catalytic C-terminal domain (CTD), in which we have ectopically expressed a truncated TbCatL reporter without the CTD (TbCatLΔ). TbCatL silencing is lethal and is rescued by TbCatLΔ. TbCatLΔ localizes properly to the lysosome and is synthesized as P and X precursors that are processed normally in the lysosome (prodomain removal). Precursor X is formed with an ER-retained TbCatLΔ reporter, but not with catalytically inactive TbCatLΔ (C150A) mutant, indicating that X forms by autocatalytic cleavage of newly synthesized precursor in the ER. Modeling the TbCatL prodomain on the human CatL structure suggests the N-terminus is composed of two α-helices (α1, α2) separated by a loop (L1). L1 has been reported to contain innate signals (K47, G49, K50) for post-Golgi trafficking of the *T. cruzi* orthologue [Huete-Perez et al. (1999) JBC 274:16249]. Mutation of these residues did not affect TbCatLΔ processing and targeting to the lysosome, nor did mutation of conserved residues (E34, E35) in the α1 helix. Collectively these results indicate that the L1 loop and α1 helix are not involved in the post-Golgi sorting of TbCatL. Current work is focused on conserved elements in the α2 helix.

81 Trypanosoma cruzi infection alters host central carbon metabolism and increases glucose uptake for potential parasite utilization

Shah-Simpson, Sheena (Harvard T. H. Chan School of Public Health, Boston, MA, USA); Lentini, Gaelle (Harvard T. H. Chan School of Public Health, USA); Dumoulin, Peter (Harvard T. H. Chan School of Public Health, USA); Burleigh, Barbara (Harvard T. H. Chan School of Public Health, USA)

Intracellular pathogens have been shown to target host central carbon metabolism to improve nutrient access or modulate the host immune response. Previous transcriptomic and functional screens in *Trypanosoma cruzi* infected cells highlight host energy metabolism pathways as important for parasite survival and intracellular growth. Here we used the small molecule ELQ300 to selectively inhibit *T. cruzi* mitochondrial respiration in infected monolayers and revealed that host mitochondrial respiration is increased during infection, with a corresponding increase in host mitochondrial mass specifically within infected cells. However, this increase in mitochondrial respiration is dispensable for parasite growth. As the host mitochondrial response to infection resembles glucose starvation, we turned our attention to host glucose metabolism, expecting to find decreased glucose uptake and glycolytic rates in infected cells. Instead, we found that infection dramatically increases glucose uptake without altering host glycolytic rates, as assessed by Seahorse extracellular flux measurements and lactate assays. We also demonstrated that exogenous glucose is important in supporting intracellular parasite growth. Because free glucose is predicted to be limiting in mammalian cell cytosol where *T. cruzi* amastigotes replicate, it is thought that they rely on other carbon sources for fuel. However, freshly isolated intracellular amastigotes take up labeled 2-deoxyglucose and respond to glucose addition by increasing rates of extracellular acidification and oxygen consumption, indicating the ability to utilize glucose for energy generation in this life cycle stage. Together, our results suggest that intracellular infection with *T. cruzi* amastigotes drives increased host glucose transport, which may be utilized by the amastigotes as an energy substrate to fuel growth. Ongoing studies using radiolabeled and fluorescent glucose analogues will resolve whether intracellular parasites are able to directly access glucose *in situ*.

82 Reconstitution and purification approaches to study architecture of multiprotein RNA editing complexes in trypanosomes

Wang, Hong (Boston University, Boston, MA, USA); Afasizheva, Inna (Department of Molecular & Cell Biology, Boston University Henry M. Goldman School of Dental Medicine, USA); Afasizhev, Ruslan (Department of Molecular & Cell Biology, Boston University Henry M. Goldman School of Dental Medicine, USA)

Uridine (U) insertion/deletion editing is a complex post-transcriptional RNA processing pathway that occurs in mitochondria of kinetoplastid protists. RNA editing is mediated by trans-acting guide RNAs and often adds and deletes hundreds of uridines to generate translatable mRNA. Two principal multiprotein complexes, RNA editing core (RECC) and RNA editing substrate binding (RESC), and other accessory factors are responsible for catalyzing and regulating RNA editing process. Previous studies identified a network of RNA-mediated co-complex and intra-subunit protein-protein interactions. However, the molecular architecture and mechanism of RNA recognition by RESC and assembly of the RNA editing holoenzyme remain unclear. Here, we explore rapid affinity purification and *in vitro* reconstitution approaches to obtain samples for cryoelectron microscopy. The RECC complex and a guide RNA binding module from the RESC complex (GRBC1/2), have been purified from *T. brucei* and *E. coli*, respectively. Furthermore, we reconstituted and isolated GRBC1/2 heterotetramer with bound guide RNA. Ultimately, we aim to resolve high-resolution structures of RECC and RESC complexes by combining mass spectrometry and cryoelectron microscopy.

83 In vivo cleavage specificity of Trypanosoma brucei editosome endonucleases

Carnes, Jason (Center for Infectious Disease Research, Seattle, WA, USA); McDermott, Suzanne (Center for Infectious Disease Research, USA); Anupama, Atashi (Center for Infectious Disease Research, USA); Oliver, Brian G. (Center for Infectious Disease Research, USA); Sather, D. Noah (Center for Infectious Disease Research, USA); Stuart, Kenneth (Center for Infectious Disease Research, USA)

RNA editing is an essential post-transcriptional process that creates functional mitochondrial mRNAs in Kinetoplastids. Multiprotein editosomes catalyze pre-mRNA cleavage, uridine (U) insertion or deletion, and ligation as specified by guide RNAs. Three functionally and compositionally distinct editosomes differ by the mutually exclusive presence of the KREN1, KREN2 or KREN3 endonuclease and their associated partner proteins. Because endonuclease cleavage is a likely point of regulation for RNA editing, we elucidated endonuclease specificity *in vivo*. We used a mutant gamma ATP synthase allele (MGA) to circumvent the normal essentiality of the editing endonucleases, and created cell lines in which both alleles of one, two or all three of the endonucleases were deleted. Cells lacking multiple endonucleases had altered editosome sedimentation on glycerol gradients and substantial defects in overall editing. Deep sequencing analysis of multiple RNAs from such cells revealed clear discrimination by editosomes between sites of deletion versus insertion editing and preferential but overlapping specificity for sites of insertion editing. Thus, endonuclease specificities *in vivo* are distinct but with some functional overlap. The overlapping specificities likely accommodate the more numerous sites of insertion versus deletion editing as editosomes collaborate to accurately edit thousands of distinct editing sites *in vivo*.

84 Outer-kinetochore phosphatase function suggests a conserved mode of attachment regulation in trypanosomes

Brusini, Lorenzo (University of Nottingham, Nottingham, GBR); Wickstead, Bill (University of Nottingham, USA)

Faithful genome segregation during mitosis relies upon regulated interactions between the spindle and newly duplicated chromosomes. Kinetochores form this attachment and reversible phosphorylation of specific components ensures chromosome bi-orientation at metaphase and sister chromatid separation at anaphase. In particular, in model systems a phosphorylation gradient formed by the centromeric Aurora B kinase and outer kinetochore PP2A and PP1 phosphatases selectively corrects erroneous microtubule attachments. Mitosis in trypanosomes differs from canonical models in a number of ways. In *Trypanosoma brucei*, despite the need to segregate ~120 chromosomes, fewer than 10 kinetochore-like structures are visible during mitosis and components of the kinetochore have very little similarity to those outside of kinetoplastids. We recently identified a set of proteins present at the trypanosome outer kinetochore. One of these, KKIP7, is a phosphoprotein phosphatase that is specifically enriched at the *T. brucei* kinetochore during metaphase. Here, we show that KKIP7, while not present in model systems, is found in several eukaryotic lineages in addition to Kinetoplastida. This family is characterised by N-terminal extensions not present in most phosphoprotein phosphatases, but phylogenetic inference suggests the catalytic domain is closely related to PP1/PP2A families. Disrupting KKIP7 function through displacement of native KKIP7 by a phosphatase-dead form severely reduces parasite proliferation and causes defects in completion of mitosis. These data suggest that regulation of kinetochore attachment in trypanosomes involves spatially separated kinase-phosphatase antagonism highly similar to that seen in models, and that the KKIP7 family may have a role in mitosis in other systems.

85 Probing the nutritional requirements and essential metabolic functions of the trypanosomatid lysosome

Young, Simon A. (University of St. Andrews, St. Andrews, GBR); Smith, Terry (University of St Andrews, USA)

Current treatments for kinetoplastid related diseases are inadequate and there is an urgent need for lead compounds that can be translated into safe, cheap, and easy to administer drugs. The causative agent of Human African Trypanosomiasis, *Trypanosoma brucei*, relies upon the endocytosis from the mammalian bloodstream of macromolecules such as lipids and proteins. Proper functioning of the lysosome, the terminal compartment of the endocytic pathway, is critical for digestion of these macromolecules generating metabolites vital for the proliferation and survival of *T. brucei*. However, little is known about this organelle with only a handful of proteins characterised. To understand the catabolic activities of the lysosome in more detail we are assessing the specific nutritional requirements of this highly metabolically active pathogen when cultured with bovine serum. In order to study essential processes and identify new drug targets within the *T. brucei* lysosome, we have utilised a non-digestible macromolecule to more easily purify the organelle from the subcellular fraction. From the acid phosphatase positive particles, characteristic of lysosomes, obtained we have identified known lysosomal markers and proteins with a range of digestive, structural and transport related functions. Lipidomic analyses of the particles has revealed an unusual content of predominantly saturated fatty acids with an altered phospholipid profile relative to the rest of the cell. This approach is allowing us to investigate lysosomal catabolic processes in more detail and assess compounds that will disrupt the function of this essential *T. brucei* organelle.

86 The ciliary gate: how are intraflagellar transport proteins concentrated at the flagellum base?

Jung, Jamin (Trypanosome Cell Biology Unit, Institut Pasteur, INSERM U1201, Paris, France, USA); Santi-Rocca, Julien (Trypanosome Cell Biology Unit, Institut Pasteur, INSERM U1201, Paris, France, USA); Fort, Cècile (Trypanosome Cell Biology Unit, Institut Pasteur, INSERM U1201, Paris, France, USA); Perrot, Sylvie (Trypanosome Cell Biology Unit, Institut Pasteur, INSERM U1201, Paris, France, USA); Bastin, Philippe (Trypanosome Cell Biology Unit, Institut Pasteur, INSERM U1201, Paris, France, USA)

Trypanosoma brucei is a protozoan parasite responsible for sleeping sickness in Sub-Saharan Africa. Trypanosomes possess flagella, important organelles required for motility and cellular signalling, found in a brought range of eukaryotes. Flagellum formation is dependent on Intraflagellar Transport (IFT) in order to transport building blocks to the site of assembly at the flagellum tip. IFT proteins are concentrated in a IFT pool found at the base of the flagellum, from where multiprotein complexes (IFT trains) travel bidirectional within the organelle.

We show that a single knock-out of IFT172 leads to halved protein amount per cell, but does not impair the amount of IFT proteins in the flagellum itself. This leads to the question of how IFT proteins are concentrated in the IFT pool and how this pool is maintained?

High-resolution microscopy revealed that the pool of IFT proteins forms a donut-like structure that colocalises with the transitional fibers that anchor the basal body to the flagellum membrane. Due to colocalisation of IFT proteins with YL1/2, we identified the retinitis pigmentosa *Tb*RP2 protein as a potential candidate in IFT pool maintenance.

Here we report that RNAi knock-down of *Tb*RP2 impairs the concentration of IFT proteins at the flagellum base prompting an important role in this process. Loss of *Tb*RP2 results in loss of the IFT pool and affects IFT trafficking, consequently leading to cells close to an IFT knock-down phenotype, with construction of shorter flagella. We propose that *Tb*RP2 acts as a ciliary gate preventing that IFT proteins diffuse out of the IFT pool into the cytoplasm.

87 Physicochemical optimization of lapatinib analog NEU-1953 for human African trypanosomiasis drug development

Ferrins, Lori (Northeastern University, Boston, MA, USA); Klug, Dana (Northeastern University, Allston, MA, USA); Bag, Seema (Northeastern University, Boston, MA, USA); Singh, Baljinder (Northeastern University Department of Chemistry, USA); Tanghe, Scott (New York University School of Medicine, USA); Bachovchin, Kelly (Northeastern University, Boston, MA, USA); Rodriguez, Ana (New York University School of Medicine, USA); Thomas, Sarah M. (University of Georgia, Athens, GA, USA); Wiedman, Justin (University of Georgia, Department of Cellular Biology, USA); Sharma, Amrita (University of Georgia, Department of Cellular Biology, USA); Mensa-Wilmot, Kojo (University of Georgia, Athens, GA, USA); Sciotti, Richard J. (Experimental Therapeutics, Walter Reed Army Institute for Research, USA); Pollastri, Michael P. (Northeastern University, Boston, MA, USA)

Lapatinib, an approved drug for breast cancer treatment, was identified through a target class repurposing campaign as a proliferation inhibitor of *Trypanosoma brucei*, the causative agent of human African trypanosomiasis (HAT). A hit-to lead medicinal chemistry campaign resulted in compound NEU-1953 which had good potency, aqueous solubility, and selectivity over mammalian cells, but was rapidly metabolized. A variety of well-established strategies were employed to optimize the overall ADME profile of NEU-1953, resulting in compounds with improved potency against *T. brucei* and significantly better pharmacokinetic properties. One such compound, NEU-4438, is a bona fide lead drug in a mouse model of HAT. Future efforts include the application of these strategies on other lapatinib-based scaffolds that show activity against different human parasites, such as *Trypanosoma cruzi*, *Leishmania* spp and *Plasmodium falciparum*.

POSTERS: Session B
Tuesday 7:00 pm

04/25/17

88 Mutagenesis screens identify editosome protein domains that differentially affect RNA editing between life cycle stages of Trypanosoma brucei

McDermott, Suzanne M. (Center for Infectious Disease Research, Seattle, WA, USA); Carnes, Jason (Center for Infectious Disease Research, USA); Stuart, Kenneth (Center for Infectious Disease Research, USA)

Uridine insertion and deletion RNA editing generates functional mitochondrial mRNAs in *Trypanosoma brucei*. Transcripts are differentially edited in bloodstream form (BF) and procyclic form (PF) cells, correlating with changes in mitochondrial function between life cycle stages. The mechanisms controlling developmental regulation are unknown. Editing is catalyzed by three distinct ~20S editosome complexes, whose composition appears to be identical in BF and PF. However, novel random and targeted mutagenesis screens of editosome proteins revealed there are intrinsic functional differences between BF and PF editosomes. The three editosomes contain a common set of 12 proteins, but differ in the mutually exclusive presence of the KREN1, N2 or N3 endonucleases and their respective KREPB8, B7, or B6 partner proteins, which are in proximity to the endonucleases and have no known catalytic functions. We hypothesize that the endonucleases and their partner proteins are likely key points of regulation for RNA editing. We generated BF and PF cell lines that are conditionally null for KREPB6-8, and show that these proteins are essential for editing in both stages. We used unbiased random mutagenesis, and targeted mutagenesis of predicted domains, in these BF and PF cell lines to screen for substitutions that disrupt protein function in each life cycle stage. Thus, our mutagenesis screens identify functional editosome protein domains, and domains that can differentially affect RNA editing between life cycle stages of *Trypanosoma brucei*.

89 RNA-binding protein capture reveals a diversity of poly(A)+ RNA-binding proteins in insect-form Trypanosoma brucei

Fisher, Michael (Rutgers - New Jersey Medical School, USA); Das, Anish (Rutgers - New Jersey Medical School, USA); Bellofatto, Vivian (Rutgers - New Jersey Medical School, USA)

The vast majority of the genome of the deadly human parasite *Trypanosoma brucei* is constitutively transcribed. Post-transcriptional mechanisms are therefore primarily responsible for regulating dynamic gene expression patterns throughout the *T. brucei* life cycle. *T. brucei* must be metabolically nimble to survive its diverse insect vector and mammalian host environments. In the insect vector, survival likely requires a unique set of mRNA-protein interactions to coordinate post-transcriptional gene regulation. Briefly, we used zero-distance UV-crosslinking to covalently fasten proteins to RNAs in live *T. brucei* insect-form cells, stringent oligo d(T)-based isolations to enrich for poly(A)+ RNA-protein complexes, and mass spectrometry to identify RNA-binding proteins (RBPs). We detected 1,216 proteins that reproducibly interact with poly(A)+ RNAs. Experimentally-validated and predicted RBPs are highly represented in this *T. brucei* insect-form RBP dataset, and 377/1,216 (31%) are also present in the *T. brucei* bloodstream-form RBP dataset. Furthermore, there is significant overlap between yeast, worm, human, and *T. brucei* insect-form RBP orthologs, suggesting evolutionary conservation of a core set of RBPs. We are currently validating our dataset by investigating a subset of our *T. brucei* insect-form RBPs that are unique to trypanosomes, previously unassociated with RNA-binding activity, and highly consequential for parasite fitness. We have confirmed that *in situ* TY1-tagged translational fusions of these proteins are produced in *T. brucei* insect-form cells in *in vitro* culture and we have proceeded to the optimization of protein-RNA binding assays. We will ultimately determine the RNA targets of these proteins, how these proteins alter transcript stability or affect translation rates, the effects of these RBPs on *T. brucei* biology, and the potential for these RBPs to serve as drug targets.

90 High-efficiency chemical-mediated transfection of Trypanosoma cruzi

Olmo, Francisco (London School of Hygiene and Tropical Medicine, USA); Rotger, Carmen (Universitat de les Illes Balears, USA); Taylor, Martin C (London School of Hygiene and Tropical Medicine, USA); Costa, Fernanda C (London School of Hygiene and Tropical Medicine, USA); Costa, Antonio (Universitat de les Illes Balears, USA); Kelly, John M (London School of Hygiene and Tropical Medicine, USA)

Electroporation is the only reported method for transfection of *Trypanosoma cruzi*. However, the procedure has limited efficiency and flexibility, taking up to 6 weeks to establish a genetically transformed culture. Here, we describe chemical-mediated transfection of this parasite using a toxicity-free squaramide-based macrocycle. As proof of principle, we used a 5.7 kb episomal plasmid which facilitates the expression of RFP. The squaramide macrocycle is able to efficiency pack the DNA construct into globular particles of less than 200 nm diameter (visible under atomic force microscopy), with a zeta potential charge of +25 mV. These physicochemical properties enable the particle to be readily internalized without damaging the parasite or disrupting the cell cycle. The procedure is straightforward: packaging of the DNA involves 40 minutes incubation at room temperature, with uptake by the parasite requiring a further 2 hours incubation with the packaging mix. Parasites expressing RFP are visible within 6 hours and they can be cultured under drug selection drug to generate stably transformed lines. In combination, with other improvements in transfection efficiency, we have been able to generate >3000 stable transformants per microgram of plasmid DNA.

91 Proteomic analysis of cell-cycle regulation in Trypanosoma brucei

Benz, Corinna (Lancaster University, USA); Urbaniak, Michael D. (Lancaster University, Lancaster, GBR)

In the divergent eukaryote *Trypanosoma brucei* the cell cycle is highly organised, coordinating nuclear and kinetoplast DNA replication and the segregation of single copy organelles. Gene expression occurs in polycistronic units and is regulated post-transcriptionally, causing the abundance of a subset of mRNAs to vary across the cell cycle [1]. The genome contains many identifiable cell cycle regulators, but the signalling mechanisms coordinating the post-transcriptional regulation of the cell cycle are largely undefined.

Here, we describe our efforts to use quantitative mass spectrometry to observe changes in protein and phosphorylation site abundance in cell cycle synchronised populations, focusing on the dynamic phosphorylation of RNA binding proteins. The experimental approach combines SILAC isotopic labelling [2] with counter-flow centrifugal elutriation to obtain synchronised cell populations. We have optimised the centrifugal elutriation so that pure G1 fractions of procyclic form cells can be isolated in a single run. The cells proliferate with unchanged kinetics and remain tightly synchronised through multiple cell-cycles [3]. We have also achieved successful synchronisation of bloodstream form cells by centrifugal elutriation for the first time. We report on our efforts to validate temporal proteomic profiles with experimental observations in order to identify novel cell cycle regulators in the parasite.

[1] Archer et al. (2011) PLoS One, 6, e18425.

[2] Urbaniak et al. (2015) *J. Proteome Res*, 12, 2234.

[3] Benz et al. (2017) *Manuscript in preparation*.

92 Identification of a microtubule-binding kinetochore protein in Trypanosoma brucei

Akiyoshi, Bungo (University of Oxford, Oxford, GBR)

Faithful transmission of genetic material is essential for the survival of all organisms. Eukaryotic chromosome segregation is driven by the kinetochore, the macromolecular protein complex that assembles onto centromeric DNA and captures spindle microtubules during mitosis and meiosis. Although it was widely assumed that the core kinetochore consists of proteins that are common to all eukaryotes, we recently identified 20 kinetochore proteins (KKT1–20) in *Trypanosoma brucei* and discovered that they constitute kinetochores unique to kinetoplastids. We are currently characterizing the function of these unconventional kinetochore proteins to reveal fundamental principles of the eukaryotic segregation machinery. Although the majority of KKT proteins are essential for accurate chromosome segregation based on their knockdown analysis, none of the 20 KKT proteins we have identified to date has significant similarity to known microtubule-binding proteins (such as Ndc80, Dam1, Ska1, EB1, and PRC1) at the primary sequence level. It therefore remained unknown which KKT protein (if any) binds microtubules. To address this question, we performed in vitro microtubule sedimentation assays using recombinant KKT proteins. We identified a protein that robustly co-sedimented with microtubules in vitro via a domain that does not have a similarity to any known microtubule-binding domain, raising a possibility that the unconventional kinetoplastid kinetochore interacts with spindle microtubules in a distinct manner compared to other eukaryotes. I will discuss insights obtained from our latest experiments.

93 RNAi library screening reveals a panel of novel T. b. brucei apolipoprotein-L1 sensitivity determinants, but no role for ICP/cathepsin-L

Currier, Rachel (London School of Hygiene & Tropical Medicine, USA); Cooper, Anneli (University of Glasgow, USA); Burrell-Saward, Hollie (London School of Hygiene & Tropical Medicine, USA); MacLeod, Annette (University of Glasgow, USA); Alsford, Sam (London School of Hygiene & Tropical Medicine, London, GBR)

Apolipoprotein-L1, the toxic component of human serum trypanolytic factors (TLFs), confers innate immunity against most African trypanosomes (*T. b. gambiense* and *rhodesiense*, have evolved distinct apoL1-resistance mechanisms). TLF1 enters *T. b. brucei* by receptor-mediated endocytosis via the haptoglobin-haemoglobin receptor (HpHbR). The acidic pH in the endo-lysosomal network causes apoL1 to undergo a conformational change, enabling membrane integration and lysosomal-mitochondrial membrane permeabilisation, leading to cell death. While the mechanisms of TLF1 uptake, apoL1 membrane integration, and *T. b. gambiense* and rhodesiense apoL1-resistance are well characterised, our understanding of the range of factors that drive apoL1 action in *T. b. brucei* is incomplete. We previously identified four sensitivity determinants following human serum RNAi library selection, including TbHpHbR and inhibitor of cysteine protease (ICP). To identify factors that specifically influence apoL1 action, we selected our *T. b. brucei* RNAi library with apoL1, revealing 63 putative sensitivity determinants. This protein set included the known factors, kinesin TbKIFC1 (links lysosomal-mitochondrial membrane permeabilisation) and V-ATPase (drives endo-lysosomal acidification), as well as an array of novel sensitivity determinants, including six putative ubiquitin-dependent proteins (at least one of which has an epistatic interaction with TbKIFC1), and a number of proteins putatively involved in membrane homeostasis and transport. Intriguingly, our screens and subsequent analyses revealed that ICP loss has no effect on apoL1 sensitivity, indicating that, in contrast to TLF, free apoL1 is not vulnerable to intracellular cathepsin-L attack. Our findings highlight a complex network of proteins that influences apoL1 action, and have implications for our understanding of TLF's anti-trypanosomal action.

94 TrypTag: Genome-wide protein localisation in the trypanosome

Billington, Karen (University of Oxford, USA); Halliday, Clare (University of Oxford, USA); Madden, Ross (University of Oxford, USA); Dean, Samuel (University of Oxford, USA); Sunter, Jack (University of Oxford, USA); Wheeler, Richard (University of Oxford, USA)

Trypanosomes are exquisitely ordered and structured cells in which protein localisation can be extremely informative of function; both for conserved eukaryotic biology and specific parasite biology. Therefore, using high throughput endogenous gene tagging, we aim to systematically localise all proteins encoded in the trypanosome genome by both N- and C-terminal fluorescent protein tagging. Here, we will present a genome-wide dataset from the first phase of the project. As of January 2017, after a first pass of the genome, we have successfully generated N-terminal tagging cell lines for 70% of the 7168 proteins without a signal peptide. ~75% gave clear localisations. The localisations appear reliable, with control proteins matching known localisations, ~80% agreement between N and C-terminal tagging (n=196), and 60 to >90% agreement with published organelle proteomes. *T. brucei* is the first flagellated organism to have a pan-genomic localisation of proteins, and is the second organism ever (after yeast) to have all its proteins localised. Unique genome-wide cohort analysis is now possible. Localisation images and annotation are publicly available immediately after annotation on a dedicated website (www.tryptag.org) and is being incorporated into TriTrypDB.

SD, JS and RW are co-Principal Investigators on the TrypTag project along with Keith Gull, Mark Carrington, Sue Vaughan and Christiane Hertz-Fowler. The TrypTag project is funded by the Wellcome Trust as a Wellcome Trust biomedical resource grant 108445/Z/15/Z.

95 Lipid rafts and dynamic palmitoylation in protozoan parasite Trypanosoma brucei

Sharma, Aabha (Northwestern University, Chicago, IL, USA)

Trypanosoma brucei, *Trypanosoma cruzi* and *Leishmania sp* employ different strategies for surviving within the mammalian host. While several proteins have been identified as virulence factors in each organism, little is known about how these factors may be regulated, for example, by post translational modifications. One such post translational modification common to all eukaryotes is S-palmitoylation, the reversible addition of palmitic acid to cysteine residue of a protein by a palmitoyl acyl transferase (PAT) enzyme. Dynamic palmitoylation then occurs by removal of this moiety by an acyl protein thioesterase (APT) enzyme. The reversible nature of this modification allows palmitoylated proteins to be shuttled on and off the membrane, thereby regulating membrane dependent function of APT substrates. We characterized the single APT in *T. brucei*, which is most similar to the human APT2 identified in a lipid raft proteome of *T. brucei* procylic parasites. Indeed the biochemical properties of TbAPT and human APT 2 are similar. Both knockdown and overexpression of TbAPT resulted in decreased parasite virulence in mice, despite there being no notable *in vitro* phenotype. Since TbAPT is a single acyl-thioesterase in an organism with 12 PATs, it is likely that a defect in proper protein palmitoylation homeostasis mediates the virulence defect and that improper regulation of specific protein palmitoylation is responsible. We are currently identifying substrates of TbAPT to further understand dynamic palmitoylation in *T. brucei*.

96 Metacyclogenesis of Leishmania is halted in the absence of proline alanine transporter

Schlisselberg, Doreen (Center for Infectious Disease Research, USA); Koren Nitzan, Roni (Technion-Israel Institute of Technology, USA); Iankovici, Liat (Technion-Israel Institute of Technology, USA); Inbar, Ehud (National Institutes of Health, USA); Zilberstein, Dan (Technion-Israel Institute of Technology, Haifa, ISR)

Leishmania parasites have developed mechanisms of adaptation to the distinct environments of host and vector that favor utilization of proline and alanine. *L. donovani* proline-alanine transporter, LdAAP24, is the major supplier for the cellular pools of proline and alanine, plays an important role in parasite osmoregulation and expressed exclusively in promastigotes. The two identical and adjacent copies of *LdAAP24* give rise to two variants; one produce the full-length transporter (LdAAP24.2), the second is 18 amino acids shorter (LdAAP24.1). LdAAP24.2 translocates proline and alanine whereas LdAAP24.1 translocates only proline. Null mutants lacking *LdAAP24* cascade are unable to develop to metacyclic-like cells in culture. These mutants express metacyclic-specific genes such SHERP and meta-1 earlier than wild type. Whereas wild type parasites express these genes at stationary phase, *Δldaap24* express SHERP and meta-1 transiently at mid-log phase, not in culture that parallel metacyclic like cells (e.g. late stationary phase). These observations implied that *Δldaap24* mutants experience some sort of earlier aging of the culture, likely due to their compromised pool of free amino acid. Interestingly, whereas log phase promastigotes express the two variant of LdAAP24, in stationary phase only the short variant is expressed. Our results indicate that LdAAP24 N-terminus play a role in enabling metacyclogenesis of *Leishmania* promastigotes. This study indicate for the first time that amino acids (e.g. proline and alanine) play a role in Leishmania development to metacyclics.

97 Interaction of the Trypanosoma brucei cap-binding protein EIF4E2 with a histone-mRNA binding protein homolog

Freire, Eden (Instituto Aggeu Magalhaes/FIOCRUZ, USA); Bezerra, Maria Jose (Instituto Aggeu Magalhaes/FIOCRUZ, USA); Vashisht, Ajay (USA); Zamudio, Jesse (USA); Sharp, Philip (USA); Rezende, Antonio Mauro (Instituto Aggeu Magalhaes/FIOCRUZ, USA); Wohlschlegel, James (USA); Sturm, Nancy (University of California, Los Angeles, USA); Campbell, David (University of California, Los Angeles, USA); de Melo Neto, Osvaldo P. (Instituto Aggeu Magalhaes/FIOCRUZ, Recife, Pernambuco, BRA)

In higher eukaryotes, the selective recruitment of mRNAs by the translation machinery is an important event associated with different mechanisms controlling gene expression. A critical participant in this process is the heterotrimeric initiation complex, eIF4F, formed by the eIF4E, eIF4G and eIF4A subunits. eIF4E, the cap binding protein, binds to the cap structure found in the 5' end of mature mRNAs and is essential for mRNA selection during translation initiation. Six eIF4Es are found in Trypanosomatids (named EIF4E1 to 6), with EIF4E3 and EIF4E4 more likely to be functioning in general translation, while EIF4E1 may have a role in translation repression and EIF4E5 and EIF4E6 seem to be involved in mRNA processing steps. EIF4E2 is less well known, but it is one of the least abundant of the eIF4Es and does not bind to other eIF4F-like subunits. Here we have investigated the *Trypanosoma brucei* EIF4E2 and found that it is a non-essential protein that binds to mature mRNAs having a cap4. We also report the description of a new partner for EIF4E2, identified as one of two putative Histone mRNA binding proteins (HBP) whose genes are found in Trypanosomatids. This protein, named here HBP2, shares a conserved mRNA binding domain found in typical HBPs but is unrelated to the HBP2 found in metazoans. The two Trypanosomatid HBPs bind to the same set of mRNAs but only HBP2 is specifically associated with EIF4E2. Both EIF4E2 and HBP2, in contrast to HBP1, are differentially expressed during cell culture, suggesting a potential role associated with selective mRNA recognition and translation regulation during specific stages of the parasite life cycle.

98 The Divergent Small Tim Homologues are Essential for Import and Assembly of TbTim17 and Other Polytopic Mitochondrial Inner Membrane Proteins in Trypanosoma brucei

Smith, Joseph T. (Meharry Medical College, Nashville, TN, USA); Singha, Ujjal (Meharry Medical College, USA); Misra, Smita (Meharry Medical College, USA); Chaudhuri, Minu (Meharry Medical College, USA)

The small Tim proteins belong to a group of mitochondrial intermembrane space chaperones that aid in the import of mitochondrial inner membrane proteins with internal targeting signals. *Trypanosoma brucei* possesses at least three small Tim proteins that include homologues of Tim9 (TbTim9), Tim10 (TbTim10), and a third small Tim that shares homology with both Tim8 and Tim13 (TbTim8/13). We found that these three small TbTims are expressed as soluble mitochondrial proteins. Small TbTims are also associated with TbTim17 and a fraction of these proteins is present in a similar size protein complex as TbTim17. TbTim9 and TbTim10 directly interact with TbTim8/13 very strongly but interact with each other very weakly, which is in contrast to the interaction pattern of small Tims in other eukaryotes. In addition, small TbTims also directly interact with TbTim17 and stronger interaction was found between TbTim17 and TbTim8/13. Small TbTims are essential for cell growth and critical for the import and assembly of mitochondrial ADP/ATP carrier (AAC), a canonical substrate for these chaperones. Interestingly, depletion of any of the small TbTims severely reduces the levels of TbTim17, the major component of the TIM complex in *T. brucei*, post-transcriptionally. Further studies revealed that although import of TbTim17 into mitochondria was partially reduced, its assembly into the larger protein complexes was greatly hampered due to knockdown of the small TbTims. Altogether, our results revealed that the divergent small TbTims possess a different intermolecular interaction pattern and play a crucial role in the biogenesis of TbTim17, as well as other polytopic mitochondrial inner membrane proteins.

99 New insights into the biosynthetic pathway of VSGs in Trypanosoma brucei

Jones, Nicola G. (Biozentrum der Universität Würzburg, USA); Reimer, Anastasija (Biozentrum der Universität Würzburg, USA); Stigloher, Christian (Biozentrum der Universität Würzburg, USA); Engstler, Markus (Biozentrum der Universität Würzburg, USA)

African trypanosomes are covered with a dense coat of variant surface glycoprotein (VSG) on their cell surface. The integrity of this coat is essential for protecting the parasite from the host immune system: (a) by forming a dense barrier and thus minimising access to the cell surface, (b) by being mobile which allows clearance of bound antibodies through a hydrodynamic flow-driven process, and (c) by exchanging the VSG exposed on the cell surface with one of the many functional VSGs encoded in the genome or with a newly-generated mosaic VSG. In order to fulfil these protective functions the VSG needs to be produced and transported efficiently through the biosynthetic pathway. In addition, it must be able to readily traverse the endosomal compartment during the constant recycling of the VSG from and to the cell surface.

While screening a range of different VSG mutants, we came across one mutant that led to a pre-cytokinesis cell cycle arrest following induction of expression. In addition, electron microscopy analysis showed an accumulation of tubular structures in the posterior part of cells expressing this mutant. Correlative light and electron microscopy revealed that the mutant VSG was present in these tubules whereas the endogenous VSG, which could not be cleared from the cells due to the cell cycle arrest, was not.

We hypothesise that these tubules are a natural component of the biosynthetic pathway of VSGs *en route* to the flagellar pocket, most likely located between the Golgi and endosomal compartments, and that they become readily detectable in electron micrographs due to accumulation caused by the faulty cargo.

100 Two Novel Components of Trypanosoma brucei Mitochondrial Inner Membrane Translocase, Tim62 and Tim54, Possess Different Substrate Specificities

Singha, Ujjal (Meharry Medical College, USA)

Ujjal K. Singha**,** Aparajita Saha, Minu Chaudhuri*
Department of Microbiology and Immunology, Meharry Medical College, Nashville TN 37208 [* Corresponding author]

The translocase of the mitochondrial inner membrane (TIM) imports a majority of the nucleus-encoded proteins those are destined to the matrix, inner membrane (IM) and the intermembrane space (IMS). In opisthokonts, Tim17, Tim23, and Tim22 are three homologous core components of the two TIM complexes, Tim23-17 and TIM22-54, which are specific for imports the N-terminal and a special class of internal mitochondrial targeting signal (MTS)-containing proteins, respectively. Interestingly, trypanosomatids possesses a single homologue of this protein family, TbTim17. How this single protein performs such a diverse function is not clear. We showed that TbTim17 forms a modular type protein complex and associates with several novel trypanosome-specific Tims. Here, we further characterized the functions of two such novel proteins, TbTim62 and TbTim54. TbTim62 is an integral inner membrane (IM) protein but TbTim54 is a peripherally associated IM protein exposed in the IMS. Blue-Native gel electrophoresis revealed that TbTim17 is present in multiple complexes within the range of ~150 to ~1100 kDa. TbTim62 and TbTim54 are found in the larger complex of ~1100 kDa. TbTim62 is also present in the smaller complex of ~150 kDa and is involved in the assembly of TbTim17. *In vitro* and *in vivo* protein import assays revealed that TbTim17 is required for import of both the N-terminal and internal MTS-containing proteins. TbTim62 knockdown only inhibits import of the N-terminal while TbTim54 is primarily involves in the import of internal signal-containing mitochondrial carrier family proteins (MCPs). Together, our data suggests that TbTim17 and TbTim62 are the part of the presequence translocase and TbTim54 in association with TbTim17 plays an important role in translocation of the MCPs in *T. brucei*.

101 Molecular Characterization of Trypanosoma cruzi TcVps26-Like

Rubio Ortiz, Margarita (Molecular Biomedicine Department, CINVESTAV-IPN, USA); Martínez Calvillo, Santiago (UBIMED, FES-Iztacala, UNAM, USA); Manning Cela, Rebeca Georgina (Molecular Biomedicine Department, CINVESTAV-IPN., USA)

During its complex life cycle, *Trypanosoma cruzi* is exposed to several microenvironmental changes that induce its differentiation process. However, very little is known about the molecular mechanisms involved in the differentiation of the parasite. In this work we characterized a sequence (TcCLB.506941.210) obtained in a subtraction library between an intermediary form (IF) and trypomastigotes. The *in silico* analysis of TcCLB.506941.210, annotated as a putative protein in the TritrypDB database, indicates that this sequence contains a pfam03643 domain, which is present in Vps26 proteins that are part of the retromer complex. Moreover, its predicted tridimensional structure is almost identical to the crystal structures of mouse and human Vps26. Therefore, we named TcCLB.506941.210 sequence as TcVps26-Like. Quantitative RT-PCR analysis showed differential level of TcVps26-Like mRNA in IF (100%) in comparison with epimastigotes (17%), trypomastigotes (33%) and amastigotes (63%). TcVps26-Like knock-down (KD) parasites expressing an antisense sequence (57 bp) against TcVps26-Like mRNA were generated. Transfected parasites showed a decrease of 94% in the level of TcVps26-Like mRNA. Notably, KD parasites showed a decrease of 47% in their efficiency to differentiate extracellularly from epimastigotes into trypomastigotes and amastigotes. Moreover, primary infection studies showed that the KD parasites have a severe deleterious effect in their invasive (99.9% at 48 hours post-infection) and infective (97% at 15 days post-infection) capacity to target cells, with the consequent decrease in the number of released parasites (99.9% at 15 days post-infection) into the supernatant of infected cells. All these results suggest that TcVps26-Like has a role in the differentiation and invasion processes of *T. cruzi*.

102 Comparative studies of the T. brucei malate dehydrogenase isoforms

Springer, Amy L. (UMass Amherst, Amherst, MA, USA)

Malate dehydrogenase (MDH) is an important enzyme that catalyzes the oxidation of oxaloacetate to malate, concomitant with the reduction of NAD+ to NADH. It highly conserved and many organisms contain several isoforms, MDH function is important not only as part of the citric acid cycle reactions but also as part of the malate aspartate shuttle used to maintain redox balance. The wealth of structural and functional information about MDH from model organisms is used to inform studies of the orthologs from a range of evolutionarily distinct organisms, currently being conducted by a consortium of collaborating faculty from various colleges and universities. This collaboration is a means of providing authentic research experiences in a teaching laboratory setting. *Trypanosoma brucei* is known to have three functional MDH isoforms localized to mitochondria, cytoplasm or glycosomes, respectively. Biochemical analyses of recombinant trypanosome MDHs have been used to provide senior Biochemistry and Molecular Biology undergraduates at UMass Amherst with authentic research projects in a required laboratory course, and are being carried out in parallel with similar analyses being performed on MDH orthologs at other universities. We have undertaken the cloning of each isoform in different expression vectors, in the presence of absence of C- or N-terminal tags, and expression studies to determine optimal conditions. These studies provide tools for further studies of biochemical and kinetic properties of the *T. brucei* enzymes and to perform comparative analyses with well characterized mammalian and plant MDH enzymes.

103 Essential or redundant? Dissection of flagellar pocket protein function in African trypanosomes

Whipple, Sarah (University of Nottingham, Nottingham, GBR); Gadelha, Catarina (University of Nottingham, USA)

To survive in the host bloodstream, *Trypanosoma brucei* must perform the critical cellular processes of nutrient uptake and secretion whilst evading the host immune response. These functions are performed at the flagellar pocket, making this the primary interface between the parasite and its host. The overall importance of the pocket for parasite virulence, and the essentiality of the few receptors characterised to date, make flagellar pocket proteins attractive therapeutic targets. Our lab has recently characterised the cell surface proteome of bloodstream-form *T. brucei*. Here we present work towards the functional and biochemical characterisation of sixteen novel flagellar pocket components identified in this surfeome. The majority of these components are glycosylated, possessing both high mannose and low mannose/complex N-glycans, but there is no clear correlation between type or number of N-glycans and localisation. Similarly, the mechanism of protein sorting to the flagellar pocket appears independent of conserved protein topology or specific sequence motifs. Ablation of individual proteins by RNA interference in both nutrient-rich and simplified growth media resulted in a growth defect for just one of the sixteen genes, suggesting that despite being located in a privileged site, the function of most individual receptors is dispensable for parasite growth in culture.

104 Some peculiarities of kinetoplastid lipid metabolism

SMITH, TERRY (UNIVERSITY OF ST ANDREWS, USA); YOUNG, SIMON (UNIVERSITY OF ST ANDREWS, USA); MAJOR, LOUISE (UNIVERSITY OF ST ANDREWS, USA); DENTON, HELEN (UNIVERSITY OF ST ANDREWS, USA); ROBERTS, MATTHEW (UNIVERSITY OF ST ANDREWS, USA); CORON, ROSS (UNIVERSITY OF ST ANDREWS, USA); DICKIE, EMILY (UNIVERSITY OF ST ANDREWS, USA)

The membranes of kinetoplastids contain a complex array of lipids that are *de novo* synthesized from precursors obtained either directly from the host, or as catabolised endocytosed lipids, while some utilise unmodified host lipids.

Their rapid cell division and trimmed down biosynthesis, make some of them an ideal organism to try and decipher and understand how a cell maintains lipid homeostasis.

The development of novel tools allows lipidomic and metabolic assessments of chemically and genetically manipulated parasites to be phenotyped in detail.

There are a number of peculiar aspects of lipid metabolism in various kinetoplastids that will be highlighted, underlining the rich seam of potential drug targets and therapeutic interventions in this area.

105 Mapping of the binding sites of naphthalene-based inhibitors on Trypanosoma brucei RNA editing ligase 1

Mehta, Vaibhav (McGill University, USA); Moshiri, Houtan (University of Toronto, USA); Salavati, Reza (McGill University, USA)

The X-ray crystal structure of *Trypanosoma brucei* RNA editing ligase 1 (*Tb*REL1) in complex with ATP, paved the way for the discovery of C35 (V2) and V4 naphthalene based inhibitors of the adenylylation step by competing with ATP for the active site (Durrant *et al*, PlosNTD 2010; Moshiri *et al*, JBC 2010). While these compounds inhibit the *in vitro* adenylylation step of recombinant protein *Tb*REL1 (r*Tb*REL1) in a 1-10 mM range, they interfere with editosome-RNA interactions in the context of native *Tb*REL1 in purified editosomes (Moshiri *et al*, JBC 2010 for C35; unpublished for V4). The differences that we observed between the effects of compounds in the recombinant and native *Tb*REL1, underscores their low efficiency. In fact, when we measured the inhibition constants (Ki) against r*Tb*REL1, the equilibrium constants were 500-1000 times larger than the dissociation equilibrium constant (Kd) for ATP (12 nM), suggesting that the lower affinities for r*Tb*REL1 are one of the reasons for off-targeting. In this work, by varying the predicted binding-site residues of r*Tb*REl1, we determined which residues are important for inhibitor activity. While F209, R288 and R309 appear to be crucial for C35 interaction with the ligase, K87, V88, F209 and R309 are important for V4 binding. Our data suggests partially overlapping binding sites for the two inhibitors with distinct contributions from the residues surrounding the active site of *Tb*REL1. These results provide an important guideline for the design of more potent and specific *Tb*REL1 inhibitors.

106 Lipidomics analysis of the intracellular human parasite, Trypanosoma cruzi, reveals potential sources of lipid scavenging from host cells.

Gazos-Lopes, Felipe (Harvard T.H. Chan School of Public Health, USA); Martin, Jessica (Harvard T.H. Chan School of Public Health, USA); Burleigh, Barbara (Harvard T.H. Chan School of Public Health, USA)

Trypanosoma cruzi is the causative agent of Chagas disease, which affects over 8 million people. Little is understood about how this parasite interfaces with its host environment and how it co-opts host metabolism to fulfill its nutrient requirements for growth. Previously, we reported the results of a genome-wide functional screen that implicated host fatty acyl scavenging by *T. cruzi* as a key factor supporting intracellular growth of this parasite. To better appreciate the potential requirements and mechanisms for parasite fatty acid scavenging from host cells, we conducted a comparative lipidomics analysis of this parasite and different mammalian host-cells. Here, we identified and quantified 1718 lipid species belonging to more than 20 different lipid classes. Overall, the lipidomes of intracellular amastigotes grown in different host cell-types were remarkably conserved, despite there being considerable differences between the lipidomes of the two host cell-types analyzed in this study. These conserved features included a clear enrichment of triacylglycerides (TGs) in amastigotes when compared to their cognate host cells. Interestingly, the fatty acyl composition of amastigote TGs closely mirrored the host cell TG profile, even though the fatty acyl composition of the remainder of its lipidome did not. To test the hypothesis that host TG pools were a source of fatty acyl scavenging by this parasite, we traced the incorporation of exogenously-derived pentadecanoic acid into different lipid classes in amastigotes and host-cells. Our results suggest that host TGs are the main source of fatty acyl scavenging by amastigotes. Future studies will be aimed at elucidating the molecular mechanisms involved in TG acquisiton in this system, focusing on experiments using host-cells with different metabolic backgrounds.

107 The role of de novo myo-inositol synthesis and metabolism in Leishmania parasites

Liu, Tim Chung-Ting (University of Melbourne, USA); Sansom, Fiona (University of Melbourne, USA); McConville, Malcolm (University of Melbourne, USA)

Leishmania are dependent on *myo*-inositol for the synthesis of the bulk membrane lipid, phosphatidylinositol, free and protein-linked glycosylphosphatidylinositol (GPI), and organelle-specific phosphoinositides. In order to investigate the extent to which *L. mexicana* promastigotes and amastigotes are dependent on the canonical pathway of *de novo myo*-inositol synthesis versus salvage from the extracellular milieu, we have generated knock-out lines lacking the two enzymes in this pathway, inositol 3-phosphate synthase (INO1) and inositol 3-phosphate monophosphatase (IMP). As expected, the *L. mexicana* Δ*ino1* mutant was unable to synthesize *myo*-inositol *de novo* (from ^{13}C-glucose labeling experiments) and was strictly dependent on exogenous *myo*-inositol for normal growth in culture. This mutant proliferated normally in *ex vivo* infected macrophages, although it was avirulent in BALB/c mice, indicating potential differences in nutrient levels in cultured and lesion macrophages. In contrast, deletion of IMP did not lead to disruption of *de novo myo*-inositol synthesis or *myo*-inositol auxotrophy, indicating redundancy in this step or that IMP has another function. Strikingly, the *L. mexicana* Δ*imp* mutant was unable to proliferate in macrophages or cause lesions in mice. Current studies are being directed towards understanding the function of the IMP gene/enzyme and the identification of the enzyme that catalyzes the second step in *de novo myo*-inositol synthesis.

108 The trypanosome outer kinetochore complex

D Archivio, Simon (University of Nottingham, Nottingham, GBR); McDonald, Jennifer (University of Nottingham, USA); Wickstead, Bill (University of Nottingham, USA)

At mitosis, replicated chromosomes are segregated by the microtubules of the spindle through an interaction mediated by the kinetochore. In most organisms, kinetochores are built around a site of specialized chromatin, the centromere. Centromeric chromatin recruits a set of ~16 proteins known as the constitutive centromere-associated network (CCAN), which forms the core of the inner kinetochore. The CCAN in turn recruits the outer kinetochore, including the Ndc80 complex, which binds to spindle microtubules. Mitosis in African trypanosomes is unusual in that they segregate a very large number of chromosomes with fidelity and lack readily identifiable homologues of most model kinetochore proteins. We recently reported the identification of KKIP1, a protein distantly related to Ndc80 and Nuf2. KKIP1 is present at the trypanosome outer kinetochore and is essential for spindle stability and chromosome segregation, suggesting that despite high divergence, the trypanosome kinetochore is derived from a machinery common to all eukaryotes. Affinity purification after limiting, reversible cross-linking showed that KKIP1 interacts with previously described kinetochore proteins (KKTs) and a new set of proteins, KKIPs, present at the outer kinetochore. Here, we describe the composition and boundaries of this outer kinetochore complex by mapping the interactions between each component. We show by affinity purification that in the absence of cross-linking KKIP2-6 form a stable complex with two newly identified outer kinetochore components. Most are loaded to the kinetochore at G2, but KKIP5 is lost at anaphase in a proteasome-dependent manner. One of the new interactors is a canonical poly(A)polymerase specifically localised to kinetochores at mitosis, forming an unanticipated link between chromosome segregation and the gene expression regulation machinery in trypanosomes.

109 The trypanosome flagellum transition zone is a bidirectional conduit that operates in the absence of the flagellar pocket

Dean, Samuel (University of Oxford, Oxford, GBR); Gull, Keith (University of Oxford, USA)

Trypanosomes have a single flagellum that emerges through the lumen of an invagination of the plasma membrane called the flagellar pocket. The biology of the trypanosome flagellum provides opportunities for insights into trypanosome pathology and can be generalised to other eukaryotic flagella.

The flagellum transition zone (TZ), positioned between the distal end of the basal bodies and the proximal end of the 9+2 axoneme, has increasingly become recognised as of central importance to flagellum growth and function. The transition zone and associated appendages are generally thought to act as a ciliary gate keeper, maintaining the different composition of the flagellum and cell body that is required for development and signaling. Many of the human inherited ciliopathy diseases are now recognised as transition zone diseases.

We previously identified dozens of new transition zone proteins (TZPs) that localise to distinct compartments within the TZ (Dean et al. 2016. Cilium transition zone proteome reveals compartmentalization and differential dynamics of ciliopathy complexes Proc Natl Acad Sci USA 113: E5135–43. doi:10.1073/pnas.1604258113). Most TZPs show long term stable association with the flagellum and several were shown to have essential roles in building flagellar structures.

Here, we use the TZP cohort and combine it with HaloTag labeling and reporter cell lines to understand how the TZ operates as a selective conduit to control the composition of the flagellum. We show that access of surface membrane proteins to the flagellum membrane is selective. Moreover, we provide evidence that transport of populations of flagellum membrane proteins across the TZ is bidirectional and that the TZ boundary remains intact even after biogenesis of the flagellar pocket is disrupted.

110 Different classes of RNA require distinct Mex67 paralogs for processing and nucleocytoplasmic export in trypanosomes.

Obado, Samson (The Rockefeller University, New York, NY, USA); Zhang, Wenzhu (The Rockefeller University, USA); Brillantes, Marc (Rutgers, New Jersey Medical School, USA); Field, Mark (University of Dundee, USA); Chait, Brian (The Rockefeller University, USA); Rout, Michael (The Rockefeller University, USA)

The nuclear pore complex (NPC) is at the center of nucleocytoplasmic transport as well as many aspects of gene regulation. A series of steps involving the NPC enable the translation of genes to proteins, starting with the initiation of transcription, through quality control and transport through the NPC, to finally the remodeling of ribonucleoprotein (RNA-protein) complexes on the cytoplasmic face of the NPC. We recently described the architecture of the trypanosome NPC. Notably absent from the cytoplasmic face of the trypanosome NPC are a host of anchored RNA export and remodeling proteins found in the opisthokont (yeast and human) cytoplasmic RNA export platform. This pointed to a significantly different mode of RNA export and processing in trypanosomes. An evolutionarily conserved group of proteins termed nuclear export factors (NXFs) are responsible for the transport of bulk mRNA from the nucleus to the cytoplasm for translation into proteins, as well as aiding ribosomal subunit export. There is a single major nuclear export factor in yeast (Mex67 or NXF1 in metazoa); although metazoans have additional tissue-specific NXF variants. All variants of NXF form a heterodimer with Nuclear Transport Factor 2 (NTF2)-Like Export Factor 1 (NXT1 in humans or Mtr2 in yeast), which facilitates NPC localization and translocation. Orthologs of both NXF and NXT have been identified in trypanosomes, termed TbMex67 and TbMtr2 respectively. Our data suggests that there are in fact *three* Mex67 paralogs in trypanosomes. We provide evidence strongly indicating they have crucial differing functions. As RNA processing and export are highly integrated events, the export pathway is likely an important step in the regulation of gene expression in trypanosomes.

111 Expression site associated genes are not essential for Trypanosoma brucei survival in vitro

Chamberlain, James (The University of Nottingham, Nottingham, GBR); Gadelha, Catarina (University of Nottingham, UK, USA)

In bloodstream-form *Trypanosoma brucei*, the active *VSG* gene is co-transcribed with a set of expression site associated genes (*ESAG*s). There are 14 families of ESAG-encoded proteins, most of which have been shown to localise to the cell surface. Alongside *ESAG*s in the expression sites, the families encompass genes-related to *ESAG*s (*GRESAG*s) that are present at chromosomal internal and subtelomeric locations. Despite their presence in the expression site (ES), the function of the majority of ESAGs remains unknown. To investigate the essentiality of ESAG families, and to disentangle the roles played by GRESAG counterparts, ESAGs in the active ES were GFP-tagged to enable specific knockdown of ES-encoded ESAGs without affecting GRESAGs. RNA-interference against *ESAG*s in the active ES produced no defects in growth in culture, in spite of good levels of protein ablation. In contrast, knockdown of both ESAG and GRESAG copies with pan-family RNAi recapitulated previously observed loss-of-fitness. These findings strongly suggest that GRESAGs provide a substantial contribution to (GR)ESAG family function in bloodstream-form cells, and that although most *(GR)ESAG* families are required for parasite survival, surprisingly the ES copy is not. This deepens the question as to why *ESAG*s are present at these privileged genomic locations in *T. brucei*.

112 Targeted Approach for Trypanomiasis Theraphy : Hexokinase in the Crosshair

Suryadi, Jimmy (Clemson University, USA)

Trypanosoma brucei bloodstream form parasites rely on glycolysis for energy. The *T. brucei* hexokinase 1 (TbHK1) gene is essential for parasite viability and has low sequence similarity with human glucokinases, supporting the idea that TbHK1 could be a potential target for trypanosomiasis treatment.

Using heterologous expression of recombinant parasite hexokinases (HKs), we have screened small molecule collections to identify inhibitors, yielding compounds with promising activity against the enzymes and parasites. Through an optimized HTS campaign, a benzamidobenzoic acid inhibitor was identified. Development of this scaffold yielded a probe (ML 205) that had encouraging potency (< 1 μM) against TbHK1 enzyme activity, but lacked efficacy against the bloodstream form trypanosome. Structural modifications of ML205 have improved *in vivo* activity, yielding potential leads for future development.

In our effort to improve understanding of structure-activity relationship of the drugs and resolve the drug inhibition mode, we are using cryo-EM and molecular modeling to elucidate the structure of TbHK1. Gel filtration and native gel analysis have demonstrated that the protein forms oligomers consistent with hexamers. More recently, these hexamers have been visualized by transmission electron microscopy. As a first step toward solving the structure, a low-resolution model of TbHK1 hexamers has been developed. The model suggests the TbHK1 oligomers have a torus-shaped tertiary structure.

To identify proteins that interact with TbHKs, we have expressed recombinant TbHK1 bearing a biotin ligase (BirA) fusion partner in trypanosomes. While the TbHK C-termini have been modified previously without detectable consequence, fusion partners could interfere with activity or oligomerization. To overcome this potential obstacle, constructs that have the BirA in multple positions have been developed. Preliminary western blotting of the soluble fraction of transgenic parasites suggests the successful expression of the full-length constructs. Biotinylated proteins that bind to, or are nearby, will be identified and their potential roles discussed here.

113 Molecular architecture of the axoneme of Trypanosoma brucei by cryo-electron tomography

Imhof, Simon (UCLA, USA)

Simon Imhof[1], Khanh Huy Bui[2], Hoangkim Nguyen[1], Gerasimos Langousis[1]
Wei Dong[3], Hong Zhou[1,3], Kent Hill[1,,3]
[1]McGill University Montreal
[1]UCLA, Dept of MIMG and Molecular Biology Institute
[3]California NanoSystems Institute

African trypanosomes propagate in a corkscrew-like motion using an unusual flagellar waveform that travels from tip to base, this is distinctively different from the flagellar waveform generated in other organisms. Since the trypanosome flagellum plays an important role in motility and host-parasite interaction, it is important to understand the molecular architecture of the flagellum in order to understand its function.

Recently, with advances in cryo-electron tomography, the molecular architectures of flagella in *Chlamydomonas*, *Tetrahymena*, and sea urchin sperm have been resolved at ~30-40 Angstrom. However, no high-resolution structures have been achieved for any members of the Excavata supergroup that includes basal eukaryotes such as trypanosomes. As such, we lack a full understanding of the structural diversity of the eukaryotic flagellum. Moreover, this represents a critical gap in knowledge of one of the central features of trypanosome biology. We have solved the molecular architecture of the axoneme of *Trypanosoma brucei* at ~35 Angstrom resolution by cryo-electron tomography. The axonemal structure of *T.brucei* shows gross similarity to that of other species but also remarkable differences, including alternative dynein distribution and inter-doublet linkage arrangement. Our results advance our understanding of axoneme structural diversity and reveal structural foundations for the understanding of the distinctive flagellar waveform that defines the *Trypanosoma* genus name.

114 Involvement of TcAlba30 in the stage-specific regulation of ß-amastin expression in Trypanosoma cruzi

Perez Diaz, Leticia (Facultad de Ciencias, Montevideo, URY); Smircich, Pablo (Facultad de Ciencias, UdelaR, USA); Silva, Tais (Universidad Federal de Minas Gerais, USA); Teixeira, Santuza (Universidad Federal de Minas Gerais, USA)

Amastins constitute a group of small surface glycoproteins, first identified in amastigotes of *T. cruzi* but later found to be expressed in several *Leishmania* species, as well as in *T. cruzi* epimastigotes. Amastin differential expression results from regulatory mechanisms involving changes in mRNA stability and/or translational control. Although distinct regulatory elements were identified in the 3′ UTR of *T. cruzi* and *Leishmania* amastin mRNAs, to date only one RNA binding protein in *L. infantum* (LiAlba20) was demonstrated to bind to the 3′ UTR of a δ-amastin mRNA contributing with stage-regulated stability of amastin transcripts. Here we investigated the role of TcAlba30, the LiAlba20 *T. cruzi* ortholog, in the post transcriptional regulation of amastin genes. TcAlba30 protein is expressed in all stages of the *T. cruzi* life cycle. Transfected parasites expressing a cMyc tagged TcAlba30 were generated. RNA immunoprecipitation using anti-Myc antibody followed by RT-PCR revealed not only TcAlba30/β-amastin RNA interaction but also altered amastin steady state mRNA levels in epimastigotes. Analysis of changes in the parasite transcriptome resulting from ectopic TcAlba30 expression in epimastigotes revealed that this protein also modulates steady state mRNA levels from other genes that co-localize in the same chromosomal region. Work is in progress to identify others TcAlba30 mRNA targets through RNA immunoprecipitation assays and sequencing.

115 Exploring the bioenergetics of the bloodstream T. brucei mitochondrion

Panicucci, Brian P. (Biology Centre ASCR, Institute of Parasitology, Ceske Budejovice, CZE); Taleva, Gergana (Biology Centre ASCR, Institute of Parasitology, USA); Ielanskyi, Mykyta (University of South Bohemia, Faculty of Science, USA); Zikova, Alena (Biology Centre ASCR, Institute of Parasitology, USA)

Our current understanding of the bioenergetics in bloodstream form (BF) *T. brucei* revolves around the ATP/ADP carrier (TbAAC) transporting cytosolic ATP generated from glycolysis into the mitochondrion, where it is hydrolyzed by F_oF_1-ATPase to maintain the essential mitochondrial (mt) membrane potential. Interestingly, BF parasites tolerate high doses of carboxyatractyloside (CATR), a specific inhibitor of AAC. Therefore, we explored the necessity of this transporter by generating BF TbAAC double knockout (DKO) cell lines. These mutants display no growth phenotype when grown in culture and they maintain almost 85% of the parental mt membrane potential. Furthermore, we have measured a modest increase in the expression levels of two enzymes that could contribute to substrate level phosphorylation inside the mitochondrion. While we are currently exploring if there is an alternative mt ATP carrier that can compensate for the loss of TbAAC, we reevaluated a subunit of succinyl CoA synthetase (TbSCoAS), an enzyme that generates ATP and had previously been shown to be essential in BF parasites. However, we again were able to generate TbSCoAS DKO cells that also don't demonstrate any significant growth phenotype. We have determined that these cells have increased TbAAC expression and are now very sensitive to CATR. This suggests that these two proteins can compensate for each other in rich media, so we are characterizing the depletion of TbAAC in TbSCoAS DKO cell lines. Furthermore, it will be interesting to determine if the loss of fitness measured in culture for both DKO cell lines will be amplified in a mouse model.

116 Blood stream form Trypanosoma brucei regulates intracellular glucose levels in a density dependent manner

Qiu, Yijian (aEukaryotic Pathogens Innovation Center Department of Genetics and Biochemistry Clemson University Clemson SC 29634, USA); Voyton, Charles (Department of Chemistry and Biochemistry Brigham Young University Provo UT 84604, USA); Christensen, Kenneth (Department of Chemistry and Biochemistry Brigham Young University Provo UT 84604, USA); Morris, James (Eukaryotic Pathogens Innovation Center Department of Genetics and Biochemistry Clemson University Clemson SC 29634, USA)

The African trypanosome, *Trypanosoma brucei*, has evolved mechanisms to communicate with the environment and regulate metabolic responses to reflect the differences in available nutrients in the mammalian host and tsetse fly vector. In the mammal, the blood stream form (BSF) depends on glycolysis to generate ATP. Two families of glucose transporters, trypanosome hexose transporter 1 (THT1) and THT2, have been described and members of each family have been subject to studies scoring kinetic behavior and gene expression patterns. Several important questions remain unresolved. First, localization studies in *Leishmania* suggest flagellar and surface localization of glucose transporters. It is unknown if the African trypanosome THTs are similarly distributed. Second, the THT1 and 2 families have multiple members, and the role of these additional THT members is unknown. Third, while it is known that the THT1s are predominant in BSF parasites while THT2 are thought to be insect stage transporters, changes in the expression of these in response to environmental glucose has not been considered in BSF parasites. Last, it remains unresolved how the regulation of the different THTs are connected to environmental cues and intracellular glucose, and the feedback from those inputs.

Here we describe the localization of the *T. brucei* THTs, assessment of expression of all of the family members, and explore the kinetics of glucose uptake by the transporters under different conditions using a glucose-sensitive probe in live parasites. Our findings suggest that BSF modulate intracellular glucose levels in a cell density-dependent fashion that is likely through altered THT expression patterns, a mechanism that points at a sophisticated regulatory mechanism.

117 Cytoplasmic RNA uridyltransferase TbTUT4 is essential in monomorphic bloodstream form of Trypanosoma brucei.

Knusel, Sebastian (Boston University, USA); Zhang, Liye (Boston University, USA); Qian, Yu (Boston University, USA); Costello, Catherine (Boston University, USA); Monti, Stefano (Boston University, USA); Roditi, Isabel (University of Bern, USA); Afasizhev, Ruslan (Boston University, USA)

Tailing of RNA with uridine residues recently emerged as a widespread mechanism of regulating gene expression in eukaryotes. Uridylation occurs on virtually all RNA species, influencing their processing and maturation, triggering degradation, or enabling translatability. Pioneering research on RNA editing in the kinetoplastid mitochondrion lead to the identification of U-tailing enzymes that defined the family of terminal uridyltransferases (TUTases). *Trypanosoma brucei* genome encodes two cytoplasmic TUTases, TbTUT3 (Tb927.10.7310) and TbTUT4 (Tb927.11.15650),

Affinity purification and proteomics analyses demonstrated that, unlike their mitochondrial counterparts, neither cytosolic TUTase forms a stable multiprotein complex. However, transient association of TbTUT3 with 3′-5′ RNA degradation machinery has been detected. Knock-outs (KO) demonstrated that the TbTUT3 gene is non-essential in procyclic Lister 427 parasites. Yet unsuccessful attempts to create conventional KO lines indicate the requirement of TbTUT3 for viability of monomorphic bloodstream forms (Lister 427 VSG 221 (TetR T7RNAP), single marker). Phenotypic analysis of conditional KO cells showed the essentiality of TbTUT4 in bloodstream forms. We are applying RNA-Seq approaches to detect transcriptome-wide changes in mRNA abundance and 3′ modification state. Our data suggest that cytoplasmic trypanosomal TUTases may be involved in developmental regulation of RNA metabolism.

118 Structural basis of prozyme activation of the major protein arginine methyltransferase in Trypanosoma brucei

Debler, Erik W. (Rockefeller University, New York, NY, USA); Kafkova, Lucie (The State University of New York at Buffalo, USA); Read, Laurie K. (The State University of New York at Buffalo, USA)

Prozymes are catalytically inactive enzyme paralogs that dramatically stimulate the function of weakly active enzymes through complex formation. We recently discovered that the major *Trypanosoma brucei* protein arginine methyltransferase, *Tb*PRMT1, functions as a heterotetrameric enzyme-prozyme pair. The inactive PRMT paralog, TbPRMT1^{PRO}, is essential for catalytic activity of the TbPRMT1^{ENZ} subunit. Here, we present the crystal structure of the heterotetrameric enzyme-prozyme pair, which elucidates its quaternary assembly, explains previous biochemical data, and provides a structural basis of prozyme activation of *Tb*PRMT1.

119 Phospholipase A2 of Trypanosoma cruzi: in silico analysis and three-dimensional structure modeling of the protein

Soares Coelho, Felipe (Federal University of Rio de Janeiro, USA); Monteiro Torres, Pedro (Federal University of Rio de Janeiro, USA); Silva, Manuela (Federal University of Rio de Janeiro, USA); Bisch, Paulo (Federal University of Rio de Janeiro, USA); Pascutti, Pedro (Federal University of Rio de Janeiro, USA); Lopes, Angela H. (Federal University of Rio de Janeiro, Rio de Janeiro, BRA)

Our group recently showed that *Trypanosoma cruzi* synthesizes a C18:1 lysophosphatidylcholine (LPC) that acts as a platelet-activating-factor (PAF). *T. cruzi* C18:1 LPC induces platelet aggregation and triggers differentiation of the parasite. LPC is synthesized by the hydrolysis of phosphatidylcholine by phospholipase A_2 (PLA_2). In this study we identified a PLA_2 gene in *T. cruzi* clone Dm28c, and built a three-dimensional structural model of the corresponding enzyme. PCR primers were designed based on the sequences of $TcPLA_2$-like from the CL Brenner strain, and of the PAF-acetyhydrolase from *Leishmania mexicana*, both available in TriTrypDB. The PCR product was sequenced and used to predict the aminoacid sequence of the Dm28c PLA_2. Using the MHOLLINE program, a 3D model was built from the alignment of the primary amino acid sequence of the putative Dm28c enzyme with that of phospholipase A_2 from CL Brenner and from various other trypanosomatids, using *in silico* tools. The 3D model of Dm28c PLA_2 closely resembled that of human PAF acetylhydrolase. Stereochemical parameters were analyzed using the software PROCHECK, and 98.1% of the residues fell within favorable and allowed regions of Ramachandran plots, thus indicating good stereochemical quality. These results may be useful for the development of more efficient and less toxic chemotherapy agents for the treatment of Chagas disease.

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120 Transcriptomic and Translatomic landscape of the Trypanosoma cruzi cell cycle.

Chavez, Santiago (Universidad de la República, Facultad de Ciencias, USA); Eastman, Guillermo (Instituto de Investigaciones Biológicas Clemente Estable, USA); Smircich, Pablo (Universidad de la República, Facultad de Ciencias, USA); Garat, Beatriz (Universidad de la República, Facultad de Ciencias, USA); Sotelo-Silveira, José Roberto (Universidad de la República, Facultad de Ciencias, USA); Duhagon, Maria A. (Universidad de la República, Facultad de Ciencias, Montevideo, USA)

Progress to the understanding of the molecular bases of the *Trypanosoma cruzi* proliferation can accelerate the development of new targeted chemotherapy to fight Chagas' disease, which remains a major health concern in Latin-American endemic regions. In order to study global gene expression regulation patterns during the progression of the parasite cell cycle, we conducted transcriptomic (RNA-Seq of poly-A RNAs) and translatomic (RNA-seq of ribosome footprints) analysis of epimastigotes (TcI) synchronized with hydroxyurea. We obtained parasite populations in G1, S and G2/M cell cycle phases (70%), as determined by cytometric measurement of parasite DNA content. The deep-sequencing of poly-A+ RNAs (30 Million mapped reads per sample) identified a set of 305 cell cycle-regulated mRNAs (CR-DEGs) (fold change ≥1.5, p-value <0.01). Transcriptome remodeling affects functions related to carbohydrates metabolism and energy production at G1-phase (70 genes), DNA and chromatin replication at S-phase (97 genes) and microtubules-based movement at G2/M-phase (138 genes). For the ribosome profiling we focus on the G1- S cell cycle transition, obtaining 1.2E+07 reads mapping on mRNAs, corresponding to 10834 genes with >10 reads. Interestingly, translational regulation affects more than 1150 genes at >2.5-fold change (CT-DEGs), 20% and 80% of which were up-regulated in G1 and S-phase respectively. Enriched molecular functions of the CT-DEGs include ribosome synthesis, nucleotide metabolism and microtubule dynamics. CR-DEGs and CT-DEGs have distinctive structural and functional properties, such as distance to the transcription start site, GC content, codon usage adaptation, gene and untranslated region (UTRs) length. Finally, a search for sequence and structural RNA motifs was attempted and the finding are presented herein. Overall, our results suggest a coordinated regulation of the periodically expressed mRNAs dependent on conserved properties of the transcripts, which relies predominantly on their translatability than their stability.

121 Understanding the TLF mediated killing of Leishmania sp.

Pant, Jyoti (The Graduate Center, City University of New York, New York, NY, USA); Keceli, Mert (USA); Nelson, Maria (USA); Raper, Jayne (The Graduate Center, CUNY, USA)

Trypanosome Lytic Factor (TLF) is an innate immunity factor originally discovered for its ability to protect against *Trypanosoma brucei* subspecies. Recently, we have shown TLF can ameliorate infections by cutaneous *Leishmania* sp. in macrophages and mice. Here, we investigated if TLF is effective at killing *Leishmania* sp. within neutrophils; one of the first cells to take up the parasites *in vivo*. We found that depleting neutrophils in TLF expressing mice exacerbates the infection indicating that TLF effectively kills parasites within neutrophils. In vitro experiments suggest that this TLF mediated killing of *Leishmania* sp. metacyclic promastigotes occurs due to the direct interaction of TLF and *Leishmania* within the acidic phagosome of phagocytic cells.

In vitro we find that metacyclics are killed by a two step process designed to mimic neutrophil uptake or macrophage uptake: TLF is first incubated in acidic media (pH 5.6 mimics the phagosome), which promotes the association of APOL1, the pore-forming protein of TLF, to the metacyclics. Then parasites are switched to neutral media to mimic the escape from neutrophil phagosomes and release into the serum. Herein, we find the parasite number is reduced but not eliminated. Alternatively if parasites are further acidified to pH 4.5 to mimic the parasitophorous vacuole of macrophages that amastigotes reside in, the metacyclics are completely lysed.

Yet *in vivo* in presence of TLF, *Leishmania* can grow and cause disease. We propose this is due to the timely differentiation to amastigotes, the form that proliferates in macrophages in vivo. We find that axenic amastigotes are completely resistant to TLF in vitro.

122 Gorilla apolipoprotein L-1 lyses T. b. brucei and is associated with both TLF1 and TLF2 in serum.

Rosa, Alessandro (USA); Raper, Jayne (USA); Verdi, Joey (USA); Ebenezer, Karen (USA); Weiselberg, Jessica (USA); Hodosh, Shana (USA); Gonzalez Baradat, Bernardo (USA)

Trypanosoma brucei sub-species have undergone a complex co-evolution with primates, leading to the emergence of trypanosome and host-specific mechanisms of infection and defense. Serum from both humans and other primates kills *T. b. brucei*. This is attributed to apolipoprotein L-1 (APOL1), which is the trypanolytic component of a subset of high-density lipoproteins (HDL) called trypanosome lytic factors (TLFs). Our understanding of this molecular arms race is limited by the lack of available non-human primate APOL1 gene sequences.

TLF1 is an HDL complex present in primates with trypanolytic sera. TLF2 is an immunocomplex comprised of an HDL and an IgM antibody that has remained undetected in non-human primates. In this study, we present evidence that TLF2 is present in Gorillas and have constructed a putative Gorilla Apol1 gene identified by sequencing genomic DNA amplicons. This synthetic version of Gorilla Apol1 was inserted into a mammalian expression vector and transfected into mice by hydrodynamic gene delivery (HGD). Sera were isolated 24 hours post-transfection and HDL was purified by density gradient centrifugation and size exclusion chromatography (SEC). Western blotting revealed that the APOL1 protein was associated with TLF1-sized lipoproteins. SEC of whole Gorilla serum revealed APOL1 detection in fractions corresponding to both TLF1 and TLF2. Moreover, *in vitro* trypanolysis assays showed that either HDL from Gorilla APOL1-expressing mice and both TLF1 and TLF2-containing fractions from Gorilla serum are able to lyse (≥85%) *T. b. brucei*. These results provide evidence that both TLF1 and TLF2 are present in Gorilla serum and that Gorilla APOL1 is the lytic component of these complexes.

123 Post-transcriptional regulation of purine transporters in the Leishmania donovani purine stress response

Licon, Haley (Oregon Health & Sciences University, USA); Martin, Jessica (Harvard School of Public Health, USA); Ortiz, Diana (Oregon Health & Sciences University, USA); Soysa, Radika (University of Washington, USA); Vanderwerf, Scott (Oregon Health & Sciences University, USA); Ullman, Buddy (Oregon Health & Sciences University, USA); Landfear, Scott (Oregon Health & Sciences University, USA); Carter, Nicola (Pacific University, USA); Yates, Phillip A. (Oregon Health & Science University, Portland, OR, USA)

As part of their lifecycles, *Leishmania* parasites must adapt to substantial fluctuations in temperature, pH, and nutrient availability within, and in the transition between, their insect and mammalian hosts. Despite its importance to survival, environmental adaptation in *Leishmania* and other kinetoplastids is poorly understood.

All protozoan parasites depend solely on salvage from their hosts to fulfill their requirement for purines, which are essential nutrients. We have used the adaptive response of *Leishmania donovani* to purine starvation as a readily tractable model of kinetoplastid nutrient stress. Induction of the purine stress response triggers an extensive remodeling of the parasite proteome. One of the earliest changes is directed at increasing the capacity of the parasites to acquire purines from the extracellular milieu, and the purine transporters NT1.1, NT2, and NT3 are among the proteins that experience the greatest increase in abundance. Our recent efforts have focused on determining how the expression of these transporters is modulated by purine availability.

Regulation of protein abundance is predominantly post-transcriptional in kinetoplastids, relying upon mechanisms affecting mRNA stability, translation rate, and post-translational protein stability. Preliminary deletion analyses have identified small positive (46 nt) and negative (33 nt) regulatory sequences in the 3'-UTRs of the *NT1.1* and *NT3* mRNAs, respectively. Using a variety of methods, we have found that purine starvation can lead to alterations in the post-translational stability of these transporters. Taken together, our results indicate that regulation of purine transporter abundance in response to purine availability is the product of transporter-specific combinations of all three modes of post-transcriptional regulation.

124 Inhibitor Screening against Trypanosoma cruzi RNA Triphosphatase

Takagi, Yuko (National Institute of Advanced Industrial Science and Technology (AIST), Japan, Tsukuba, Ibaraki, JPN); Kuwabara, Naoyuki (USA); Ho, Kiong (University of Tsukuba, USA); Sakashita, Hitoshi (USA); Furukawa, Koji (USA)

RNA triphosphatases of Protozoa, Fungi and certain viruses belong to the superfamily of triphosphate tunnel metalloenzyme (TTM). Since the structure and reaction mechanism of TTM-type RNA triphosphatases differ significantly from those of mammalian counterpart, RNA triphosphatases of protozoan parasites have been implicated as drug target. *Trypanosoma cruzi* is the etiologic agent of Chagas disease, which results in cardiac, digestive, or neurological alterations in up to 40% of infected people. Although 7 million people are estimated to carry *T*. *cruzi*, there is currently no drugs that can effectively treat chronic phase of the disease. Here we present identification of compounds that inhibit RNA triphosphatase of *T*. *cruzi*, TcCet1. We screened ~900 small molecular weight compounds by Thermal Shift Assay to first select the ones that stabilize and increase the denaturation temperature of TcCet1 upon binding. Top 21 compounds were then tested for their inhibition effect on triphosphatase activity, using ATP as substrate. We identified 10 lead compounds that inhibit TcCet1 activity, which serve as starting point for Structure-Based Drug Design against kinetoplastid RNA triphosphatase.

125 Functional analysis of TOEFAZ1 uncovers protein domains essential for cytokinesis in Trypanosoma brucei

Sinclair-Davis, Amy (Brown University, USA); McAllaster, Michael (Brown University, USA); de Graffenried, Christopher L. (Brown University, Providence, RI, USA)

The parasite *Trypanosoma brucei* is highly polarized, with a single flagellum that nucleates at the posterior of the cell and is adhered along the cell surface, extending towards the anterior. During cell division, the new flagellum attachment zone (FAZ) also functions to position the cleavage furrow, which ingresses from the anterior tip of the cell towards the posterior to produce the daughter cells. The morphogenic kinase TbPLK is essential for FAZ replication and thus cytokinesis. We recently identified a novel protein termed Tip Of the Extending FAZ 1 (TOEFAZ1) as a potential binding partner and substrate of TbPLK. Depletion of TOEFAZ1 causes a block in cytokinesis and mislocalization of TbPLK. TOEFAZ1 is comprised of three distinct domains: an N-terminal alpha helical domain, an intrinsically disordered region, and a C-terminal zinc finger. We have analyzed the localization and function of these domains using overexpression and RNAi complementation. The N-terminal domain appears to function as an oligomerization domain, while the zinc finger is essential for localization to the new FAZ tip. The IDP is not necessary for viability and its omission does not alter the localization of TOEFAZ1, although TbPLK recruitment is blocked. Our results suggest that TOEFAZ1 functions as a multimeric scaffold for recruiting proteins that control the timing and location of cleavage furrow ingression.

126 The role of rapid mitochondrial dynamics in kinetoplastid parasites

Ruthel, Gordon (University of Pennsylvania School of Veterinary Medicine, USA); DiMaio, John (Penn State Brandywine, USA); Povelones, Megan L. (Penn State Brandywine, Media, PA, USA)

Kinetoplastid organisms are powerful models for mitochondrial biology. Each cell has a single mitochondrion whose biogenesis is exquisitely coordinated with the cell cycle. Live cell studies in other eukaryotic models have established that mitochondrial dynamics, including continuous fission and fusion reactions, controls mitochondrial shape and organelle quality. While fission is ultimately required for organelle division during the cell cycle, other roles for mitochondrial dynamics in a cell with a single mitochondrion have not yet been explored. We are studying mitochondrial dynamics in *Crithidia fasciculata*. As in *T. brucei*, mitochondrial division is concurrent with cytokinesis, but in *Crithidia* these events also coincide with division of the kinetoplast DNA. Using cells expressing a mitochondrialy-targeted GFP, we have monitored the changes in organelle shape that occur during the cell cycle. As the cell doubles in size, mitochondrial tubules grow parallel to the long axis of the cell. Networks just prior to division appear hyperfused, which we hypothesize allows for homogenization of mitochondrial components. Excitingly, using live-cell imaging, we have observed that the mitochondrial network in these organisms is surprisingly dynamic even in non-dividing cells. These dynamics occur in both flagellated parasites and adhered amastigotes. Mitochondrial remodeling events such as tubule budding, outgrowth, end-to-side fusion and side-to-side fusion occur once every 3-5 minutes on average. This is similar to that observed in budding yeast, indicating that pathways for fusion and fission may be broadly conserved. The observation of remodeling outside of cell division or differentiation suggests additional roles for these events in organelle maintenance. We are currently investigating the mechanisms of kinetoplastid mitochondrial dynamics and its consequences for mitochondrial shape and function.

127 The glycobiology of African trypanosomes upon adaptation to living in adipose tissue

Rodrigues, Joao (Instituto de Medicina Molecular, USA); Wongtrakul-Kish, Katherine (Ludger, USA); Trindade, Sandra (Instituto de Medicina Molecular, USA); Pinto-Neves, Daniel (Instituto de Medicina Molecular, USA); Spencer, Daniel (Ludger, USA); Figueiredo, Luisa (Instituto de Medicina Molecular, USA)

Recent work from our group has shown that African Trypanosomes have the capacity to accumulate in large numbers in the visceral adipose tissue, where they adapt by changing gene expression of hundreds of genes (Trindade *et al*., Cell Host Microbe 2016). Two other studies also revealed that, *T. brucei* presents tropism to skin adipocytes, from which transmission can occur (Caljon *et al*., Plos Pathog 2016; Capewell *et al*., eLife 2016) suggesting that trypanosome-adipocyte interactions may somehow confer a selective advantage to *T. brucei*.

In this study, we investigated the role that host and parasite protein glycosylation play in the adaptation to living in adipose tissue. This is particularly relevant taking into consideration that *T. brucei* is absolutely dependent on a number of glycoproteins for survival and infectivity. We have used a combination of transcriptomics, proteomics and glycomics approaches to obtain a snapshot of the glycobiology of adipose tissue parasites and possible interacting host cells (adipocytes and stromal vascular fraction).

Preliminary results from our glycomics studies indicate that adipose tissue parasites contain a higher percentage of complex protein N-glycans when compared to bloodstream form parasites. In addition, several host carbohydrate binding molecules are differentially expressed (e.g. galectins), indicating that these molecules may be playing a role in modulating the host-immune response to infection.

The findings obtained will be discussed and therapeutically exploitable differences will be highlighted. Understanding the role of these galectin–sugar interactions during the course of infection and adaptation to living in different tissues might contribute to defining novel targets for disease prevention and immune intervention.

128 Crithidia fasciculata as a model to study insect-specific adhesion

Filosa, John (University of Pennsylvania, USA); Dudkin, Elizabeth (Penn State Brandywine, USA); Povelones, Megan (Penn State Brandywine, USA); Povelones, Michael (University of Pennsylvania, Philadelphia, PA, USA)

Kinetoplastids can adhere to tissues of their insect hosts, but as studies of these stages can be technically challenging, the molecules necessary for adhesion are not well understood. We are using the model *Crithidia fasciculata* to understand kinetoplastid adhesion. Mosquitoes are the natural host of *C. fasciculata*, and we can efficiently colonize both *Anopheles* and *Aedes*. *C. fasciculata* robustly colonize the lumen of the mosquito hindgut and rectal papillae, where they adhere and replicate on the apical surface of epithelial cells, forming cell clusters called rosettes. *C. fasciculata* cultures contain free-swimming flagellated forms as well as rosettes adhered to the tissue culture vessels. We asked whether *C. fasciculata* adhesion in mosquitoes and in culture are comparable. We performed transcriptomic analysis of cultured swimming forms, cultured adherent forms, and colonized mosquito hindguts. We found 877 genes that are significantly upregulated at least two-fold in both mosquito and cultured adherent cells. Gene ontology analysis shows an enrichment of genes involved in RNA metabolism, chromatin remodeling, DNA replication, and signal transduction. Amongst the upregulated genes are many with known or predicted roles in kinetoplastid-host interactions, cell adhesion, and the junctional complex. Excitingly, several are members of gene families implicated in pathogenesis in other kinetoplastid species. Taken together, our data supports *C. fasciculata* as an informative and tractable model to elucidate the mechanism of kinetoplastid adhesion, allowing studies in culture and in the insect host. We are currently conducting functional analyses to determine whether disruption of these genes affects the parasite's ability to form adherent rosettes in culture or successfully colonize the mosquito.

129 TriTrypDB: The Functional Genomics Resource for Kinetoplastids

Crouch, Kathryn (University of Glasgow, USA); Hertz-Fowler, Christiane (University of Liverpool, USA); Warrenfeltz, Susanne (University of Georgia, USA); ...on behalf of the, Kinetoplastid Database Consortium (TriTrypDB, USA)

TriTrypDB (http://TriTrypDB.org) is a free, online functional genomics database that is a component of the Eukaryotic Pathogen Bioinformatics Resource Center (http://EuPathDB.org). Development and maintenance of TriTrypDB is an ongoing collaborative effort between EuPathDB and GeneDB (http://GeneDB.org), supported by the Wellcome Trust (UK) and the NIH (US). By integrating genome sequences, annotation and functional datasets in a single site, this resource supports and expedites biological discovery and translational research on the kinetoplastida. Supported data types include the sequences and annotation for 24 species (35 strains), transcript-level information (RNA-Seq, microarray, ESTs), protein expression data, epigenomic data (ChIP-chip, ChIP-seq), population-level and isolate data (SNPs, CNV), and functional information from genome-wide RNAi knock-down analysis. The results of in-house genomic analysis pipeline are also integrated and provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (EC annotations and GO terms), and evolutionary relationships based on gene orthology (OrthoMCL). Highlights include: a whole genome annotation pipeline (Companion); a User Comment system for community annotation and curation, including images, files, PubMed records, etc, that are immediately visible through GeneDB and incorporated into TriTrypDB; a Search Strategy system for step-wise construction of complex searches which may be saved, modified and shared. (e.g. http://tritrypdb.org/tritrypdb/im.do?s=f6c4cbf89b843e8a); and statistical tools for functional enrichment analysis of query results based on Gene Ontology (GO) annotations, metabolic pathways and text terms; and KEGG metabolic pathways. Recent updates include: completely revamped gene pages, alternative transcripts represented in gene pages and strategies, new search categories on the home page with a 'Find a Search' tool, integration of TrypanoCyc and LeishCyc data, a Galaxy workspace for privately analyzing your own large scale datasets, subcellular imaging data and additional data. To learn more, visit our booth during all poster sessions.

130 Role of the RNA binding protein RBP42 in Trypanosoma brucei bloodstream form parasites

Das, Anish (Rutgers - New Jersey Medical School, USA)

Anish Das, Michael A. Fisher and Vivian Bellofatto
Department of Microbiology, Biochemistry and Molecular Genetics, International Center for Public Health, Rutgers- New Jersey Medical School, Newark, NJ

In the absence of regulated transcription, post transcriptional control plays pivotal roles in controlling gene expression in trypanosomes. RNA binding proteins, via interacting with their target mRNAs, modulate many stages of gene expression including mRNA maturation, localization, translation and decay. However, we are just beginning to understand how sets of RNA binding proteins function in these parasites to accomplish the extensive and intricately networked gene expression patterns.

Previously we have shown that an essential RNA-binding protein RBP42 mainly localize in the cytoplasm of procyclic *Trypanosoma brucei* and is tightly associated with polysomes. Using HITS-CLIP analysis, we found that in procyclic *T. brucei* RBP42 preferentially associates within the coding sequence of mRNAs involved in cellular energy metabolism.

Our current aim is to extend our knowledge of what role RBP42 plays in the metabolism of mammalian-infective bloodstream (BF) form *T. brucei*. To this end, we have generated a conditional knock-down cell line of BF *T. brucei*, in which two endogenous alleles are replaced by drug resistance marker genes and a tagged RBP42 protein is expressed from an exogenous gene cassette in a tetracycline inducible fashion. We have used quantitative proteomics analysis, using iTRAQ methodology, to determine the changes in the proteome when RBP42 levels are decreased. Our preliminary analysis suggests RBP42 depletion leads to the specific alteration of many metabolic enzyme levels in the BF parasites. These data will be presented.

131 The homologue of the yeast SNARE protein Ykt6 functions in glycosome assembly in Trypanosoma brucei

Banerjee, Hiren (University of Alberta, USA); Rachubinski, Richard A. (University of Alberta, USA)

The kinetoplastid parasites *Trypanosoma* and *Leishmania* are aetiologic agents of diseases like African sleeping sickness, Chagas disease and leishmaniasis that inflict many tropical and subtropical parts of the world. These parasites are distinctive in that they compartmentalize most of the usually cytosolic enzymes of the glycolytic pathway within a peroxisome-like organelle called the glycosome. Functional glycosomes are essential in both the procyclic and bloodstream forms of Trypanosomatid parasites, and mislocalization of glycosomal enzymes to the cytosol is fatal for the parasite. The life cycle of these parasites is intimately linked to their efficient protein and vesicular trafficking machinery that helps them in immune evasion, host-pathogen interaction and organelle biogenesis and integrity. Soluble *N*-ethylmaleimide sensitive factor attachment protein receptors (SNAREs) play important roles in vesicular trafficking and mediate a wide range of protein-protein interaction in eukaryotes. We show here that the homologue of the yeast SNARE protein Ykt6 is essential for glycosome biogenesis and function in *Trypanosoma brucei*. RNAi-mediated depletion of Ykt6 in either the procyclic form or bloodstream form of *T. brucei* leads to mislocalization of glycosomal matrix proteins to the cytosol, pronounced reduction in glycosome number, and cell death. GFP-tagged Ykt6 appears as punctate structures in the *T. brucei* cell and colocalizes in part with glycosomes. Our results constitute the first demonstration of a role for SNARE proteins in the biogenesis of peroxisomal organelles.

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Zurita-Leal, Andrea
(University of
Glasgow,USA) 158

Ruslan Afasizhev
Boston University
ruslana@bu.edu

Inna Afasizheva
Boston University
innaaf@bu.edu

Bungo Akiyoshi
University of Oxford
bungo.akiyoshi@bioch.ox.ac.uk

Sam Alsford
London School of Hygiene & Tropical Medicine
sam.alsford@lshtm.ac.uk

TAI AN
Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston
tai.an@uth.tmc.edu

Sabine Bachmaier
Biocenter, Section Genetics, University of Munich (LMU), Martinsried, Germany
sabine.bachmaier@lrz.uni-muenchen.de

Hiren Banerjee
University of Alberta
hiren@ualberta.ca

Jay Bangs
University of Buffalo
jdbangs@buffalo.edu

Hélène Baudouin
Institute of Cell Biology, University of Bern
helene.baudouin@izb.unibe.ch

Daniela Begolo
ZMBH, Heidelberg University
d.begolo@zmbh.uni-heidelberg.de

Vivian Bellofatto
Rutgers - New Jersey Medical School

Tom Beneke
University of Oxford, Sir William Dunn School of Pathology
tom.beneke@path.ox.ac.uk

Stephen M. Beverley
Washington University School of Medicine, St. Louis
stephen.beverley@wustl.edu

Michael Boshart
Faculty of Biology, University of Munich (LMU)
boshart@lmu.de

Emma Briggs
University of Glasgow
e.briggs.1@research.gla.ac.uk

Lorenzo Brusini
University of Nottingham
lorenzo.brusini@nottingham.ac.uk

James Budzak
Imperial College
j.budzak14@imperial.ac.uk

Barbara Burleigh
Harvard School of Public Health
bburleig@hsph.harvard.edu

Samantha Campbell
Wellcome Trust Centre for Molecular Parasitology/University of Glasgow
wtcmpbioinformatics@glasgow.ac.uk

Roberta Carloni
University of Edinburgh
roberta.carloni@ed.ac.uk

Jason Carnes
Center for Infectious Disease Research
jason.carnes@sbri.org

Mark Carrington
University of Cambridge, Department of Biochemistry
mc115@cam.ac.uk

Nicola Carter
Oregon Health & Science University
cartern@ohsu.edu

Mathieu Cayla
University of Edinburgh
mathieu.cayla@ed.ac.uk

Igor Cestari
Center for Infectious Disease Research
igor.cestari@cidresearch.org

James Chamberlain
The University of Nottingham
stxjwc@nottingham.ac.uk

Minu Chaudhuri
Meharry Medical College
mchaudhuri@mmc.edu

Kenneth Christensen
Brigham Young University
kenc@chem.byu.edu

Christine Clayton
ZMBH
cclayton@zmbh.uni-heidelberg.de

Raúl Cosentino
Research Center for Infectious Diseases, University of Würzburg
rulicosentino@gmail.com

Fernanda Costa
London School of Hygiene & Tropical Medicine
fernanda.costa@lshtm.ac.uk

Robbie Crickley
Oxford Brookes University
14029466@brookes.ac.uk

George Cross
The Rockefeller University
george.cross@rockefeller.edu

Kathryn Crouch
University of Glasgow
kathryn.crouch@glasgow.ac.uk

George Cruz
Texas AM AgriLife
Armando_Rey@mail.com

Marcelo da Silva
Butantan Institute
mamasantos2003@yahoo.com.br

Andreas Damianou
University of Glasgow
ad1303@york.ac.uk

Simon D'Archivio
University of Nottingham
simon.d'archivio@nottingham.ac.uk

Noopur Dave
Center for Applied Biotechnology Studies and Department of Biological Science, California State University, Fullerton, CA
noopurdave@csu.fullerton.edu

Christopher de Graffenried
Brown University
christopher_degraffenried@brown.edu

Osvaldo de Melo Neto
Instituto Aggeu Magalhaes/FIOCRUZ
opmn@cpqam.fiocruz.br

Samuel Dean
University of Oxford
samuel.dean@path.ox.ac.uk

Erik Debler
Rockefeller University
edebler@rockefeller.edu

Stephanie DeMarco
Molecular Biology Interdepartmental Doctoral Program, University of California, Los Angeles
sfdemarco@ucla.edu

Pawan Doharey
Texas A&M University
pawan.cdri@gmail.com

Gang Dong
Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria
gang.dong@meduniwien.ac.at

Maria Duhagon
Universidad de la República, Facultad de Ciencias
mduhagon@fcien.edu.uy

Peter Dumoulin
Harvard T.H. Chan School of Public Health
peter.dumoulin@gmail.com

Karen Ebenezer
Hunter College
karen5ebenezer@gmail.com

ThankGod Ebenezer
Department of Biochemistry, University of Cambridge
thankgod1980@yahoo.co.uk

Maria Echeverry
Universidad Nacional de Colombia
mcecheverryg@unal.edu.co

Nicole Eisenhuth
Department of Cell and Developmental Biology, Würzburg, Germany
nicole.eisenhuth@uni-wuerzburg.de

Markus Engstler
University of Wuerzburg
markus.engstler@biozentrum.uni-wuerzburg.de

Ben Falk
Cedars-Sinai Medical Center
ben.falk@cshs.org

Joana Faria
School of Life Sciences, University of Dundee
j.r.correiafaria@dundee.ac.uk

Mark Field
University of Dundee
mfield@mac.com

Luisa Figueiredo
Instituto de Medicina Molecular
lmf@fm.ul.pt

Rachel Findlay
University of York
rcf511@york.ac.uk

Francesca Florini
IZB, University of Bern
francesca.florini@izb.unibe.ch

Catarina Gadelha
University of Nottingham
catarina.gadelha@nottingham.ac.uk

Amit Gaurav
Cleveland State University
amitgaurav23@gmail.com

Felipe Gazos-Lopes
Harvard T. H. Chan School of Public Health
fglopes@hsph.harvard.edu

Stephanie Gomez
The George Washington University
sgomez@email.gwu.edu

Bernardo Gonzalez Baradat
Hunter College
baradatbernardo@gmail.com

Fabien Guegan
Instituto de Medicina Molecular
fguegan@fm.ul.pt

Keith Gull
University of Oxford
keith.gull@path.ox.ac.uk

Christiane Hertz-Fowler
University of Liverpool
chf@liverpool.ac.uk

Anneliese Hoffmann
University of Bern
anneliese.hoffmann@izb.unibe.ch

Galadriel Hovel-Miner
The George Washington University
ghovel_miner@gwu.edu

Simon Imhof
UCLA
simon.imhof1981@gmail.com

Daniel Jaremko
SUNY University at Buffalo
danielja@buffalo.edu

Laura Jeacock
University of Dundee
l.l.jeacock@dundee.ac.uk

Bryan Jensen
Center for Infectious Disease Research
bryan.jensen@cidresearch.org

Borka Jojic
University of Bern
borka.jojic@izb.unibe.ch

Nicola Jones
Biozentrum der Universität Würzburg
nicola.jones@uni-wuerzburg.de

Jamin Jung
Trypanosome Cell Biology Unit, Institut Pasteur, INSERM U1201, Paris, France
jamin.jung@pasteur.fr

Lucie Kafkova
Department of Microbiology and Immunology, Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of medicine, Buffalo, NY
lucie.hanzalkova@gmail.com

Murat Kalem
Biomedical Sciences, University of Minnesota Medical School, Duluth, MN, USA
muratcankalem@gmail.com

Sabine Kaltenbrunner
Biology Center, Czech Academy of Sciences, Institute of Parasitology
sabine.kaltenbrunner83@gmail.com

Anyango Kamina
University at Buffalo
dorothya@buffalo.edu

Jan Naseer Kaur
Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 14214
jannasee@buffalo.edu

John Kelly
London School of Hygiene and Tropical Medicine
john.kelly@lshtm.ac.uk

Louise Elizabeth Kerry
Imperial College London
l.kerry13@imperial.ac.uk

Hee-Sook Kim
The Rockefeller University
khee-sook@rockefeller.edu

Elizabeth King
University of St. Andrews
efk2@st-andrews.ac.uk

Michele Klingbeil
University of Massachusetts, Amherst
klingbeil@microbio.umass.edu

Sebastian Knusel
Boston University
knusels@bu.edu

Carolina Koeller
University at Buffalo
ckoeller@buffalo.edu

Nikolay Kolev
Yale University
nikolay.kolev@yale.edu

Julie Kovarova
University of Dundee
j.kovarova@dundee.ac.uk

Susanne Kramer
Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg
susanne.kramer@uni-wuerzburg.de

Amelie J. Kraus
Research Center for Infectious Diseases (ZINF), University of Wuerzburg
amelie.kraus@uni-wuerzburg.de

Emilia Kruzel
University at Buffalo, SUNY
emilia.kruzel@gmail.com

Yasuhiro Kurasawa
University of Texas Health Science Center at Houston
yasuhiro.kurasawa@uth.tmc.edu

Scott Landfear
Oregon Health & Science University
landfear@ohsu.edu

Kyu Lee
University of Texas
kyu.j.lee@uth.tmc.edu

Moara Lemos
Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, Paris, France
moara.lemos@pasteur.fr

Gaelle Lentini
Harvard School of Public Health
glentini@hsph.harvard.edu

Bibo Li
Cleveland State University
b.li37@csuohio.edu

Ziyin Li
University of Texas Medical School at Houston
ziyin.li@uth.tmc.edu

Tim Chung-Ting Liu
University of Melbourne
timl1@student.unimelb.edu.au

Angela Lopes
Federal University of Rio de Janeiro
angela.lopes@micro.ufrj.br

Julius Lukes
Biology Center
jula@paru.cas.cz

Todd Lyda
Para Tryp Research Organization
todd.a.lyda@paratryp.org

Louise Major
University of St Andrews
llm6@st-andrews.ac.uk

Catarina Marques
Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK
cmarques@dundee.ac.uk

Jessica Martin
Harvard T.H. Chan School of Public Health
jlmartin@hsph.harvard.edu

Keith Matthews
University of Edinburgh
keith.matthews@ed.ac.uk

Natalie McAdams
SUNY Buffalo
nmerlino7@gmail.com

Malcolm McConville
University of Melbourne
malcolmm@unimelb.edu.au

Richard McCulloch
University of Glasgow
richard.mcculloch@glasgow.ac.uk

Suzanne McDermott
Center for Infectious Disease Research
suzanne.mcdermott@cidresearch.org

Kirsty McWilliam
University of Edinburgh
k.r.mcwilliam@sms.ed.ac.uk

Vaibhav Mehta
McGill University
vaibhav.mehta@mail.mcgill.ca

Mikhail Mesitov
Boston University Henry M. Goldman School of Dental Medicine
mmesitov@bu.edu

Shulamit Michaeli
Bar-Ilan University
shulamit.michaeli@biu.ac.il

Amartya Mishra
The Hebrew University of Jerusalem
amartyamishra80@gmail.com

Meredith Morris
Clemson Univeristy
mmorri3@clemson.edu

Brooke Morriswood
University of Würzburg
brooke.morriswood@uni-wuerzburg.de

Florencia Mosquillo
Facultad de Ciencias, Universidad de la República
mmosquillo@fcien.edu.uy

Monica Mugnier
Johns Hopkins Bloomberg School of Public Health
mmugnier@rockefeller.edu

Sumit Mukherjee
Texas Tech University
sumit.mukherjee@ttu.edu

Laura SM Müller
Research Center for Infectious Diseases, University of Würzburg, Germany
laura.mueller@stud-mail.uni-wuerzburg.de

Peter Myler
Seattle BioMed
peter.myler@seattlebiomed.org

Vishal Nanavaty
Cleveland State University, Center for Gene Regulation in Health and Disease
vishal.nanavaty@gmail.com

Olga Nerusheva
The University of Oxford
olga.nerusheva@bioch.ox.ac.uk

Agathe Nkouawa
Yale University
agathe.nkouawa@yale.edu

Samson Obado
The Rockefeller University
sobado@rockefeller.edu

Francisco Olmo
London School of Hygiene and Tropical Medicine
francisco.olmo@lshtm.ac.uk

Cheryl Olson
Northwestern University
c-olson@northwestern.edu

Cher-Pheng Ooi
Imperial College
cher.ooi@imperial.ac.uk

Brian Panicucci
Biology Centre ASCR, Institute of Parasitology
bpanicucci@paru.cas.cz

Jyoti Pant
The Graduate Center, City University of New York
jpanta@genectr.hunter.cuny.edu

Nina Papavasiliou
DKFZ-Heidelberg and Rockefeller Univ
papavasiliou@rockefeller.edu

Diana Parra
Universidad Nacional de Colombia
dmparram@unal.edu.co

Harshal Patil
The George Washington University
harshal_patil@email.gwu.edu

Michel Pelletier
The College at Brockport
mpelleti@brockport.edu

Priscila Pena-Diaz
Czech Academy of Sciences
pena@paru.cas.cz

Doranda Perdomo
CNRS-Université Bordeaux
doranda.perdomo@u-bordeaux.fr

Leticia Pérez Díaz
Facultad de Ciencias
lperez@fcien.edu.uy

Megan Povelones
Penn State Brandywine
megan.povelones@gmail.com

Michael Povelones
University of Pennsylvania
mpove@vet.upenn.edu

Yijian Qiu
EPIC, Clemson University
yijianq@g.clemson.edu

Mckenzie Quinn
The George Washington University
quinnmr@gwmail.gwu.edu

Najma Rachidi
Institut Pasteur
najma.rachidi@pasteur.fr

Santiago Radio
UdelaR, Facultad de Ciencias, Laboratorio de Interacciones Moleculares. Ministerio de Educación y Cultura. IIBCE
sradio91@gmail.com

Julie Ralton
The University of Melbourne
julieer@unimelb.edu.au

SRINIVASAN RAMAKRISHNAN
Center for Tropical and Emerging Global Diseases
sri@uga.edu

Jayne Raper
City University of New York
jayne.raper@nyumc.org

Laurie Read
Wade J. Sigurdson
lread@buffalo.edu

Filipa Rijo-Ferreira
Instituto de Medicina Molecular
anafferreira@fm.ul.pt

Constance Rink
University at Buffalo
cirink@buffalo.edu

Sigrid Roberts
Pacific University School of Pharmacy
sroberts@pacificu.edu

Isabel Roditi
Institute of Cell biology, University of Bern
isabel.roditi@izb.unibe.ch

Federico Rojas
University of EdinburghCentre for Immunity, Infection and Evolution, Institute for Immunology and infection Research, School of Biological Sciences, A
federico.rojas@ed.ac.uk

Saúl Rojas-Sánchez
Yale School of Public Health
saul.kt@gmail.com

Gabriela Romero-Meza
Johns Hopkins Bloomberg School of Public Health
gromero5@jhu.edu

Alessandro Rosa
The Graduate Center of The City University of New Yrok
arosa@gradcenter.cuny.edu

Margarita Rubio Ortiz
CINVESTAV-IPN
mrubio@cinvestav.mx

Gloria Rudenko
Imperial College London
gloria.rudenko@imperial.ac.uk

Raquel S. Negreiros
University of Georgia
qkel20@gmail.com

Reza Salavati
McGill University
reza.salavati@mcgill.ca

Sarah Schuster
Universität Würzburg
sarah.schuster@uni-wuerzburg.de

Thomas Seebeck
University of Berne
thomas.seebeck@izb.unibe.ch

Francois Sement
Boston University
sement@bu.edu

Sheena Shah-Simpson
Harvard T. H. Chan School of Public Health
shahsimpson@fas.harvard.edu

Michal Shapira
Ben-Gurion University of the Negev
shapiram@bgu.ac.il

Aabha Sharma
Northwestern University
asharma@u.northwestern.edu

Huafang Shi
Yale School of Medicine
huafang.shi@yale.edu

Joseph Shlomai
Department of Microbiology and Molecular Genetics The Hebrew University of Jerusalem
josephs@ekmd.huji.ac.il

T. Nicolai Siegel
University of Wuerzburg
nicolai.siegel@uni-wuerzburg.de

Sara Silva Pereira
University of Liverpool
ssilva@liv.ac.uk

Eleanor Silvester
University of Edinburgh
eleanor.silvester@ed.ac.uk

Rachel Simpson
Department of Microbiology & Immunology, University at Buffalo Jacobs School of Medicine, Buffalo, NY 14214
rachel.simpson64@gmail.com

UJJAL SINGHA
MEHARRY MEDICAL COLLEGE
usingha@mmc.edu

Pablo Smircich
Facultad de Ciencias
psmircich@fcien.edu.uy

TERRY SMITH
UNIVERSITY OF ST ANDREWS
tks1@st-andrews.ac.uk

Joseph Smith
Meharry Medical College
jtsmith11@email.mmc.edu

Evan Spencer
Department of Biology
espence25@gmail.com

Amy Springer
UMass Amherst
aspringer@biochem.umass.edu

Ankita Srivastava
Department of Genetics and Genome Sciences, UConn Health, Farmington, CT
ankita26@gmail.com

C Erec Stebbins
German Cancer Research Center
e.stebbins@dkfz-heidelberg.de

Jennifer Ann Stortz
University of Glasgow
j.black.1@research.gla.ac.uk

Ken Stuart
Center for Infectious Disease Research
ken.stuart@seattlebiomed.org

Ines Subota
Universität Würzburg
ines.subota@uni-wuerzburg.de

Takuma Suematsu
Boston University
tsuemats@bu.edu

Jack Sunter
Oxford Brookes University
jack.sunter@path.ox.ac.uk

Jimmy Suryadi
Clemson University
jsuryad@clemson.edu

Yuko Takagi
National Institute of Advanced Industrial Science and Technology (AIST), Japan
yuko-takagi@aist.go.jp

Martin Taylor
London School of Hygiene and Tropical Medicine
martin.taylor@lshtm.ac.uk

Calvin Tiengwe
University at Buffalo (SUNY)
calvinti@buffalo.edu

Justin Toh
Yale University School of Public Health
justin.toh@yale.edu

Maiko Tonini
University of St Andrews
mlt4@st-andrews.ac.uk

Anna Trenaman
University of Dundee
a_trenaman@hotmail.com

Sandra Trindade
Instituto de Medicina Molecular
strindade@medicina.ulisboa.pt

Christian Tschudi
Yale School of Public Health
christian.tschudi@yale.edu

Lindsay Tulloch
Univerisyt of St Andrews
LT37@St-Andrews.ac.uk

Brianna Tylec
University at Buffalo
tylecbrianna@gmail.com

Khan Umaer
University at Buffalo
khanumae@buffalo.edu

Michael Urbaniak
Lancaster University
m.urbaniak@lancaster.ac.uk

Sue Vaughan
Oxford Brookes University
svaughan@brookes.ac.uk

Joey Verdi
Hunter College and the Graduate Center at the City University of New York
joseph.verdi05@gmail.com

Charles Voyton
Clemson University
cvoyton@g.clemson.edu

Pegine B. Walrad
Centre for Immun. and Infection, Univ. of York
pegine.walrad@york.ac.uk

Hong Wang
Boston University
wanghong@bu.edu

Susanne Warrenfeltz
University of Georgia
swfeltz@uga.edu

Carolin Wedel
Research Center of Infectious Diseases (ZINF), University of Wuerzburg, D-97080 Wuerzburg, Germany
carolin.wedel@uni-wuerzburg.de

Richard Wheeler
University of Oxford
richard.wheeler@path.ox.ac.uk

Sarah Whipple
University of Nottingham
sarah.whipple@nottingham.ac.uk

Bill Wickstead
University of Nottingham
bill.wickstead@nottingham.ac.uk

Natalie Wiedemar
Swiss Tropical and Public Health Institute
natalie.wiedemar@unibas.ch

Noreen Williams
University of Buffalo
nw1@acsu.buffalo.edu

Phillip Yates
Oregon Health & Science University
yatesp@ohsu.edu

Simon Young
University of St. Andrews
say2@st-andrews.ac.uk

Tian Yu
Boston University
tyty@bu.edu

Kai Zhang
Texas Tech University
kai.zhang@ttu.edu

Qing Zhou
University of Texas Medical School at Houston
qing.zhou@uth.tmc.edu

Dan Zilberstein
Technion-Israel Institute of Technology
danz@technion.ac.il

Sara Zimmer
University of Minnesota Medical School
szimmer3@d.umn.edu

Martin Zoltner
Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom
m.zoltner@dundee.ac.uk

Rachel Zufferey
St John's University
zufferer@stjohns.edu

Andrea Zurita
University of Glasgow
a.zurita-leal.1@research.gla.ac.uk