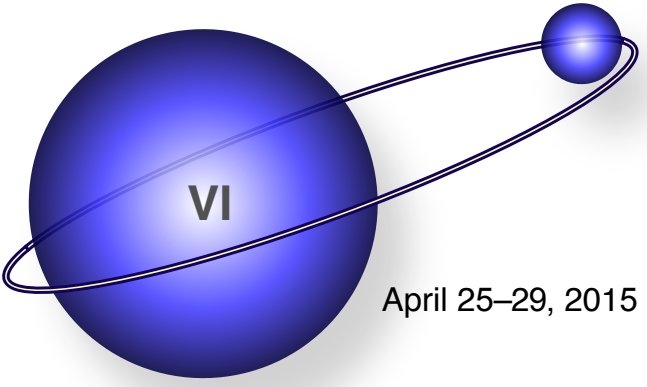
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory · Woods Hole · MA · USA



April 25–29, 2015

KMCBM 2015 Acknowledgements

Sixth Kinetoplastid Molecular Cell Biology Meeting, April 25 – 29, 2015

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 School of Public Health, Boston, USA)
Stephen M. Beverley (Washington University, St. Louis, USA)
Mark Field (University of Dundee, Dundee, Scotland, UK)
Torsten Ochsenreiter (University of Bern, Bern, Switzerland)

The Staff at MBL:

Elisabeth McCarthy 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 Sodexo Food Service at the MBL.

Cover Design: Markus Engstler

KMCBM 2015 Program

Saturday, April 25

02:00 – 05:00	Arrival, Registration and Poster Session A setup
04:00 – 06:30	Greeting and Dinner
07:00 – 09:00	Session I: Gene Expression (chair: Robert Sabatini)
09:00 – 11:00	Mixer

Sunday, April 26

07:00 – 08:30	Breakfast
08:45 – 11:45	Session II: Cell Biology I (chair: Philippe Bastin)
12:00 – 01:30	Lunch
02:00 – 04:30	Session III: Nucleus/ Kinetoplast/ Gene Expression (chair: Laurie Read)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session A
09:00 – 11:00	Mixer & Poster A/B Changeover

Monday, April 27

07:00 – 08:30	Breakfast
08:45 – 11:45	Session IV: Biochemistry/ Drugs (chair: Michael Boshart)
12:00 – 01:30	Lunch
02:00 – 03:30	Workshop
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session B
09:00 – 11:00	Mixer & Poster B/C Changeover

Tuesday, April 28

07:00 – 08:30	Breakfast
08:45 – 11:45	Session V: Pathogenesis (chair: Barbara Burleigh)
12:00 – 01:30	Lunch
02:00 – 04:45	Session VI: Elisabetta Ullu Tribute (chair: Shulamit Michaeli)
06:00 – 07:00	Dinner (Lobster)
07:00 – 09:00	POSTER PRESENTATIONS: Session C
09:00 – 11:00	Mixer

Wednesday, April 29

07:00 – 08:30	Breakfast
08:45 – 11:15	Session VII: Cell Biology II (chair: Mark Field)
12:00 – 01:30	Lunch or lunch boxes

ROOMS MUST BE VACATED BY 10:00 AM ON Wednesday

In Memoriam: Elisabetta Ullu, Ph.D.

1951-2014



Elisabetta Ullu, a brilliant scientist who made seminal contributions to the fields of RNA biology and parasitology, died on September 8, 2014, following a heroic 21-year battle with breast cancer. She was a woman who brought intelligence, style, and warmth to all aspects of her life, both personal and professional.

Elisabetta was Professor of Medicine (Infectious Diseases) and of Cell Biology at the Yale School of Medicine. An intellectual titan and talented experimentalist, Elisabetta was one of the most inspiring scientists, mentors and teachers of our time. Her influence and impact on the field of parasitology was immeasurable, not only because of her expertise but, more importantly, because of her humanity. Deeply caring and compassionate, Elisabetta was a dedicated mentor to students and scientists at all levels, especially to women scientists. Elisabetta had very high standards for what constitutes excellent science, which was clearly evident in her publications. She was interested in work far beyond her own areas of RNA biology and parasitology, and could drill down to the important question, the key data, in any scientific argument. Many junior colleagues benefited immensely from her intellectual insights. Elisabetta would not only find the weak spot in their arguments, but also turn her full attention and focus as to how that weakness could be addressed. She read their manuscripts and proposals, gave advice on navigating life as an academic scientist, and advocated ceaselessly on behalf of her junior colleagues in her section and department and beyond Yale.

“She was just such a remarkable woman. We will miss her terribly, but I feel I am a better person and scientist for knowing her.”

A comprehensive obituary is accessible at
http://publichealth.yale.edu/emd/research/ntd/genetics/ullu.aspx

SESSION I: Gene Expression
Robert Sabatini, Chair.
Saturday 7:00pm

1A The Inositol Phosphate Pathway Controls Transcription of Telomeric Expression Sites in Trypanosomes
***Cestari, Igor**; Stuart, Ken*

1B Allelic exclusion by VEX1 controls antigenic variation in trypanosomes
***Glover, Lucy**; Hutchinson, Sebastian; Horn, David*

1C An RNA-binding protein controls VSG gene silencing in African Trypanosomes
***Trenaman, Anna**; Glover, Lucy; Hutchinson, Sebastian; Horn, David*

1D Regulation of RNA Pol II transcription termination by H3V and base J modified DNA
***Reynolds, David**; Siegel, Nicolai; Beverley, Stephen; Sabatini, Robert*

1E Global transcriptome profiling of the human macrophage infected with Leishmania major, Leishmania amazonensis and latex beads
***El-Sayed, Najib M.**; Fernandes Dupecher, Cecilia; Dillon, Laura A.L.; Belew, A. Trey; Hughitt, V. Keith; Okrah, Kwame; Corrada Bravo, Hector*

1F Dynein Light Chain DYNLL1/LC8 is Essential for RNA Polymerase I Transcription in Trypanosoma brucei
***Kirkham, Justin**; Park, Sung Hee; Nguyen, Tu; Gunzl, Arthur*

1G A single-point mutation in the RNA-binding protein 6 enables progression through the complete life cycle of Trypanosoma brucei in vitro
***Shi, Huafang**; Kolev, Nikolay G.; Ullu, Elisabetta; Tschudi, Christian*

1H Tracking the in vivo dynamics of antigenic variation in Trypanosoma brucei
***Mugnier, Monica**; Cross, George A. M.; Papavasiliou, F. Nina*

SESSION II: Cell Biology I
Philippe Bastin, Chair.
Sunday 8:45am

2A The kinetoplastid parasite flagellum: the current state of our ignorance.

Gull, Keith

2B Late steps in exocytosis: Expansions, losses and novel trypanosome subunits for the exocyst

***Boehm, Cordula**; Obado, Samson; O'Reilly, Amanda; Gadelha, Catarina; Chait, Brian; Rout, Michael; Field, Mark*

2C A novel AMPKalpha1-TOR4 pathway regulates differentiation from proliferative bloodstream to quiescent stumpy form in Trypanosoma brucei

***Saldivia Concepción, Manuel Alejandro**; Navarro, Miguel*

2D Towards a first molecular model of the Tripartite Attachment Complex (TAC) and its dynamics during the cell cycle in Trypanosoma brucei

***Hoffmann, Anneliese**; Doiron, Nicholas; Trikin, Roman; Schimanski, Bernd; Jakob, Martin; Schnarrwiler, Felix; Schneider, Andre; Schnaufer, Achim; Zuber, Benoit; Ochsenreiter, Torsten*

Coffee Break

2E A new model for regulation of flagellum length in Trypanosoma brucei

***Bertiaux, Eloise**; Morga, Benjamin; Perrot, Sylvie; Rotureau, Brice; Bastin, Philippe*

2F Proteomic identification of novel cytoskeletal proteins associated with TbPLK, an essential regulator of cell morphogenesis in T. brucei

***de Graffenried, Christopher L.**; McAllaster, Michael; Ikeda, Kyojiro; Lozano-Nunez, Ana; Anrather, Dorothea; Unterwurzacher, Verena; Gossenreiter, Thomas; Perry, Jenna; Vaughan, Sue*

2G Architecture of a host-parasite interface: complex targeting mechanisms revealed through proteomics

***Gadelha, Catarina**; Zhang, Wenzhu; Wickstead, Bill; Chait, Brian; Field, Mark*

2H Exosome secretion of spliced leader RNA is a novel sensing mechanism in trypanosomes

***Michaeli, Shulamit**; Eliaz, Dror; Shaked, Hadassa; Arvatz, Gil; Tkacz, Itai Dov; Binder, Lior; Waldman Ben Asher, Hiba*

SESSION III: Nucleus/Kinetoplast/Gene Expression
Laurie Read, Chair.
Sunday 2:00pm

3A Conserved functions in the replication and segregation of nuclear and kinetoplast DNA provide a potential communication route between the two genomes in T. brucei
***Shlomai, Joseph**; Klebanov-Akopyan, Olga; Yaffe, Nurit; Glousker, Galina; Kapach, Guy; Dvir, Rotem; Porath, Danny; Tzfati, Yehuda*

3B Direct monitoring of stepwise folding of the kinetoplast DNA network
***Yaffe, Nurit**; Rotem, Dvir; Porath, Danny; Shlomai, Joseph*

3C Coupled 3'-5' degradation and 3' uridylation constitute the major RNA processing pathway in mitochondria of trypanosomes
***Afasizhev, Ruslan**; Suematsu, Takuma; Zhang, Liye; Monti, Stefano; Afasizheva, Inna*

3D Maintaining the mitochondrial membrane potential in bloodstream and dyskinetoplastic T. brucei: a game of two players.
***Zikova, Alena**; Veselikova, Michaela; Subrtova, Karolina; Panicucci, Brian*

TT1 What do kinetoplastids need a kinetoplast for? Life cycle progression of Trypanosoma brucei in the presence and absence of mitochondrial DNA
Dewar, Caroline (Poster # 69)

Coffee Break

3E Transposable elements in the Crithidia fasciculata genome: a potential role in chromosome rearrangement and transcription
***Myler, Peter J.**; Ramasamy, Gowthaman; Steinbiss, Sascha; Hertz-Fowler, Christiane; Tomlinson, Chad; Warren, Wes; Akopyants, Natalia; Beverley, Stephen*

3F Bromodomain proteins affect multiple immune evasion mechanisms and maintain life-cycle stage identity in trypanosomes
***Schulz, Danae**; Debler, Erik; Mugnier, Monica; Kim, Hee-Sook; Papavasiliou, Nina*

TT2 Ribosome profiling reveals translation control as a key mechanism generating differential gene expression in Trypanosoma cruzi
Smircich, Pablo (Poster # 70)

TT3 Development of an inducible system for Leishmania gene deletion; application to the cell cycle protein kinase CRK3
Duncan, Samuel M. (Poster # 71)

TT4 Keeping an open chromatin in the active VSG expression site is associated to uncommitted switching
Aresta Branco, Francisco (Poster # 72)

TT5 Multiple protein kinases provide diverse DNA repair-associated functions in Trypanosoma brucei.
Black, Jennifer Ann (Poster # 73)

SESSION IV:Biochemistry/Drugs
Michael Boshart, Chair.
Monday 8:45am

4A Arginine sensing pathway in Leishmania induces a Mitogen Activated Protein Kinase 2-mediated response during macrophage invasion
***Zilberstein, Dan**; Goldman, Adele; Balno, Caitlin; Strasser, Rona; Rentsch, Doris; Myler, Peter, J.; Jardim, Armando; Wiese, Martin*

4B Structural characterisation of a novel family of GPI-anchored surface glycoproteins expressed in the metacyclic stage of Trypanosoma brucei
***Acosta-Serrano, Alvaro**; Casas-Sánchez, Aitor; Fisher, Lewis; Jackson, Andrew; Boulanger, Martin*

4C Metabolic signals and glycosomal isocitrate dehydrogenase (IDHg) control procyclic to metacyclic development of Trypanosoma brucei fly stages
***Boshart, Michael**; Allmann, Stefan; Ziebart, Nicole; Dupuy, Jean-William; Bonneu, Marc; Bringaud, Frédéric; Van De Abbeele, Jan*

4D Cell surface proteomics yields insight into stage-specific remodeling of the host-parasite interface in Trypanosoma brucei
***Shimogawa, Michelle**; Saada, Edwin; Vashisht, Ajay; Wohlschlegel, James; Hill, Kent*

Coffee Break

4E Heme starvation controls proliferation and activate differentiation by inhibiting translation initiation regulation in Trypanosoma cruzi *Moretti, Nilmar; Augusto, Leonardo; Schenkman, Sergio*

4F cAMP response proteins (CARPs) regulates downstream signalling of cAMP in Trypanosoma brucei
***Tagoe, Daniel N.**; Gould, Matt; Kalejaiye, Titilola; Bachmaier, Sabine; Boshart, Michael; de Koning, Harry P.*

4G Kinetoplastid-specific pATOM36 mediates membrane insertion of a subset of mitochondrial outer membrane proteins
***Käser, Sandro**; Oeljeklaus, Silke; Warscheid, Bettina; Schneider, André*

4H Chemical biology uncovers mechanisms for controlling invariant surface glycoprotein expression in Trypanosoma brucei
***Zoltner, Martin**; Leung, Ka Fai; Alsford, Sam; Horn, David; Mark, Field*

TT6 In vivo RITseq screening for drug targets in the Trypanosoma brucei kinome *Fernandez-Cortes, Fernando (Poster # 125)*

SESSION V: Pathogenesis
Barbara Burleigh, Chair.
Tuesday 8:45am

5A Host and parasite genetics shape a link between tissue-specific Trypanosoma cruzi infection dynamics and cardiac pathogenesis

***Lewis, Michael D.**; Fortes Francisco, Amanda; Taylor, Martin; Jayawardhana, Shiromani; Kelly, John*

5B Metacyclogenesis of Trypanosoma cruzi Includes Starvation-Induced Transient Accumulation of Mitochondrial Gene Transcripts

***Shaw, Aubie K.**; Zimmer, Sara*

5C L. donovani uses a bet hedging strategy that drives evolutionary adaptation through genome instability and dosage compensation

***Späth, Gerald F.**; Prieto Barja, Pablo; Pescher, Pascale; Kedra, Darek; Chaumeau, Victor; Guerfali, Fatma; Bastien, Patrick; Sterkers, Yvon; Notredame, Cedric*

5D Genome-wide identification of negative regulators of stumpy-specific developmental gene expression

***Rico Vidal, Eva**; Glover, Lucy; Ivens, Al; Horn, David; Matthews, Keith*

5E Adipose tissue is where most Trypanosoma brucei parasites accumulate and a probable source of relapse after treatment

***Trindade, Sandra**; Figueiredo, Luisa M.; Rijo-Ferreira, Filipa; Carvalho, Tania; Van Den Abbeele, Jan; Ribeiro, Ruy M; Dias, Sergio*

Coffee Break

5F Trypanosoma brucei infection accelerates the mouse circadian clock

***Rijo-Ferreira, Filipa**; Takahashi, Joseph S; Figueiredo, Luisa M*

5G Regulating the regulators: LmjPRMT7's role in trans-regulator expression and parasite virulence

***Ferreira, Tiago R.**; Alves-Ferreira, Eliza V.C.; Defina, Tania P.; Smith, Deborah; Walrad, Pegine; Papadopoulou, Barbara; Cruz, Angela K.*

5H Association of Leishmaniavirus LRV1 with chemotherapeutic treatment failures of human leishmaniasis

***Beverley, Stephen M.**; Adaui, Vanessa; Lye, Lon-Fye; Akopyants, Natalia S.; Zimic, Mirko; Llanos-Cuentas, Alejandro; Garcia, Lineth; Maes, Ilse; De Doncker, Simonne; Dobson, Deborah E.; Arevalo, Jorge; Dujardin, Jean-Claude*

TT7 A Murine Model of Trypanosome Lytic Factor 2

Verdi, Joseph (Poster # 179)

TT8 You aren't what you don't eat: metabolic specialization is a major driver of genome evolution in kinetoplastids

Seward, Emily (Poster # 180)

SESSION VI: Elisabetta Ullu Tribute -
Shulamit Michaeli, Chair.
Tuesday 2:00pm

6A The rich repertoire of snoRNAs and the identification of dual-function snoRNAs in Trypanosoma brucei

***Michaeli, Shulamit**; Chikne, Vaibhav; Doniger, Tirza; Eliaz, Dror; Semo, Oz; Cohen-Chalamish, Smadar; Tkacz, Itai Dov; Biswas, Viplop; Kolev, Nikolay G.; Unger, Ron; Tschudi, Christian*

6B Quantitative Analysis of the Histone Acetylome and its Role in Histone Variant Deposition

***Kraus, Amelie J.**; ElBashir, Rasha; Vanselow, Jens T.; Wedel, Carolin; Janzen, Christian J.; Förstner, Konrad U.; Schlosser, Andreas; Siegel, T. Nicolai*

6C Codon usage is the major determinant of mRNA levels in Trypanosoma brucei

***de Freitas Nascimento, Janaina**; Kelly, Steve; Sunter, Jack; Carrington, Mark*

6D Biased codons control mRNA and protein abundance in the African trypanosome

***Jeacock, Laura**; Glover, Lucy; Hutchinson, Sebastian; Horn, David*

Coffee Break

6E The Functional Architecture of Trypanosoma brucei Editosomes Revealed by Cross-linking Mass Spectrometry and Random Mutagenesis

***McDermott, Suzanne M.**; Luo, Jie; Carnes, Jason; Ranish, Jeff; Stuart, Ken*

6F Novel insights into RNP granules by employing the trypanosome's microtubule skeleton as a molecular sieve

***Kramer, Susanne**; Fritz, Melanie; Vanselow, Jens; Sauer, Nadja; Lamer, Stephanie; Goos, Carina; Siegel, Tim Nicolai; Subota, Ines; Schlosser, Andreas; Carrington, Mark*

6G Identification of the First Direct Interaction between the Surface of Trypanosoma brucei and the Innate Immune System: a Factor H Receptor

***Macleod, Olivia JS**; Carrington, Mark*

6H Leishmania IFT140 mutants show normal viability but are aflagellate: a tool for the study of flagellar function through the infectious cycle

***Fowlkes, Tiffanie**; Beverley, Stephen*

SESSION VII: Cell Biology II
Mark Field, Chair.
Wednesday 8:45am

7A Complexities in coordinating morphogenesis - proximal end assembly of the flagellum attachment zone contrasts to the flagellum's distal assembly
***Sunter, Jack**; Varga, Vladimir; Dean, Samuel; Gull, Keith*

7B Components and function of the basal body transition zone in Trypanosoma brucei
***Dean, Samuel**; Gull, Keith*

7C The Trypanosoma brucei transmembrane surface protein TbGPCR-L1 induces stumpy formation through the quorum sensing signalling pathway
***Rojas, Federico**; Thompson, Joanne; Matthews, Keith R*

7D Functional characterization of small proteins in Trypanosoma brucei
***Ericson, Megan**; Obado, Samson; Kobayashi, Junya; Gould, Matthew; Schnaufer, Achim; Matsuura, Yoshiyuki; Rout, Michael; Ullu, Elisabetta; Tschudi, Christian*

7E Differential targeting to flagellum membrane sub-domains influences receptor adenylate cyclase function in Trypanosoma brucei social behavior
***Saada, Edwin A.**; Hill, Kent*

7F VSG identity and structural integrity determine growth rate of bloodstream form Trypanosoma brucei
***Schwede, Angela**; Jones, Nicola; Engstler, Markus; Carrington, Mark*

7G The role of VSG in T. brucei evasion from macrophage phagocytosis
***Geung, Jackie**; Wand, Nadina; Wheeler, Richard; Rudenko, Gloria*

POSTERS: Session A
Sunday 7:00pm

69 What do kinetoplastids need a kinetoplast for? Life cycle progression of Trypanosoma brucei in the presence and absence of mitochondrial DNA

***Dewar, Caroline**; MacGregor, Paula; Casas, Aitor; Savill, Nick; Acosta-Serrano, Alvaro; Matthews, Keith; Schnaufer, Achim*

70 Ribosome profiling reveals translation control as a key mechanism generating differential gene expression in Trypanosoma cruzi

***Smircich, Pablo**; Eastman, Guillermo; Bispo, Saloe; Duhagon, Maria Ana; Garat, Beatriz; Goldenberg, Samuel; Munroe, David; Dallagiovanna, Bruno; Holetz, Fabiola; Sotelo-Silveira, José Roberto*

71 Development of an inducible system for Leishmania gene deletion; application to the cell cycle protein kinase CRK3

***Duncan, Samuel M.**; Brewer, James; Garside, Paul; Meissner, Markus; Mottram, Jeremy*

72 Keeping an open chromatin in the active VSG expression site is associated to uncommitted switching

***Aresta Branco, Francisco**; Pimenta, Sílvia; M. Figueiredo, Luísa*

73 Multiple protein kinases provide diverse DNA repair-associated functions in Trypanosoma brucei.

***Black, Jennifer Ann**; Donatelli Serafim, Tiago; Wilkes, Jon; Alsford, Sam; Hamilton, Graham; Horn, David; Mottram, Jeremy C; McCulloch, Richard*

74 Basal body connector protein (BBCP), a basal body-specific protein interlinking with pro-basal body in Trypanosoma brucei

***LAI, De-Hua**; Moreira-Leite, Flavia; Gull, Keith*

75 Defining the mode of action of anti-trypanosomatid compounds: A next generation platform

***Moniz, Sonia**; Wall, Richard; Patterson, Stephen; Horn, David; Fairlamb, Alan; Wyllie, Susan; Wyatt, Paul; Gilbert, Ian*

76 Cutaneous and mucosal Leishmania braziliensis isolates from the same patient show differential gene expression and infection profile

***Alves-Ferreira, Eliza**; Toledo, Juliano; De Oliveira, Arthur; Ferreira, Tiago; Pinzan, Camila; Ruy, Patricia; Freitas, Ramon; Rojo, David; del Aguila, Carmen; Barbas, Coral; Barral-Netto, Manoel; Barral, Aldina; Cruz, Angela K*

77 KHARON1 Mediates Trafficking of Flagellar Membrane Proteins in African Trypanosomes and is Essential for Parasite Viability

***Landfear, Scott M.**; Sanchez, Marco; Tran, Khoa*

78 Characterization of the translationally controlled tumor protein in Trypanosoma brucei (TbTCTP)

***Jojic, Borka**; Ochsenreiter, Torsten*

79 Structural basis for ligand and innate immunity factor uptake by the trypanosome haptoglobin-haemoglobin receptor

Lane-Serff, Harriet; MacGregor, Paula; Carrington, Mark; Higgins, Matthew

80 Molecular characterization of 5S ribosomal RNA genes in Leishmania major
***Moreno-Campos, Rodrigo**; Florencio-Martínez, Luis Enrique; Manning-Cela, Rebeca; Martínez-Calvillo, Santiago*
81 Characterization of the small Tim homologues in Trypanosoma brucei
***Smith, Joseph T.**; Chaudhuri, Minu*
82 Identification of the Leishmania major U2 snRNA gene promoter
***Rojas-Sánchez, Saúl**; Florencio-Martínez, Luis Enrique; Manning-Cela, Rebeca; Martínez-Calvillo, Santiago*
83 Co-expression and interaction network analysis of host-trypanosomatid systems
***Hughitt, V. Keith**; El-Sayed, Najib*
84 Functional analysis of TbMORN1 in bloodstream form T. brucei
***Morriswood, Brooke**; Schmidt, Katy*
85 The essential roles of phosphatidylserine synthase 2 and phosphatidylserine decarboxylase in Trypanosoma brucei.
***Farine, Luce**; Bütikofer, Peter*
86 High Throughput Phenotypic Screening against Three Kinetoplastid Parasites: An Open Resource of New Compounds Sets
***PEÑA, IMANOL**; Manzano, M. Pilar; Cantizani, Juan; Kessler, Albane; Alonso-Padilla, Julio; Bardera, Ana I.; Alvarez, Emilio; Colmenarejo, Gonzalo; Rodriguez, Ana; Fiandor, Jose M.; Martin, J. Julio*
87 Molecular characterization of Maf1, putative negative regulator of RNA Polymerase III transcription, in Trypanosoma brucei
***Romero-Meza, Gabriela**; Florencio-Martinez, Luis-Enrique; Hernandez-Rivas, Rosaura; Martinez-Calvillo, Santiago*
88 Rft1 and protein N-glycosylation in T. brucei
***Gonzalez Salgado, Amaia**; Menon, Anant K.; Bütikofer, Peter*
89 Quantitative Phosphoproteomic Analysis of Stumpy to Procylic differentiation in Trypanosoma brucei
***Urbaniak, Michael D.**; Domingo-Sananes, Maria Rosa; Szoor, Balazs; Ferguson, Michael; Matthews, Keith*
90 A Novel Respiratory Protein of Trypanosomatids – A Promising Multi-Parasite Drug Target
***Menzies, Stefanie**; Fraser, Andrew; Gould, Eoin; King, Elizabeth; Tulloch, Lindsay; Zacharova, Marija; Florence, Gordon; Smith, Terry*
91 Do long ncRNAs modulate antigenic variation in Trypanosoma brucei?
***Vasquez, Juan Jose**; Siegel, T. Nicolai*

92 Conservation and function of the 70-bp repeats in Trypanosoma brucei antigenic variation

***Hovel-Miner, Galadriel A.**; Goldwater, Benjamin; Papavasiliou, F. Nina*

93 Transketolase in Leishmania mexicana: Essentiality, localisation and metabolic roles

***Kovarova, Julie**; Wildridge, David; Achcar, Fiona; Bringaud, Frederic; Barrett, Michael*

94 Target identification of anti-trypanosome compounds using an over-expression library

***Begolo, Daniela**; Erben, Esteban; Clayton, Christine*

95 Cell differentiation in Trypanosoma brucei is a bistable developmental switch requiring new protein synthesis to achieve signal memory

***Matthews, Keith R.**; Domingo Sananes, Maria Rosa; Szoor, Balazs; Ferguson, Michael; Urbaniak, Michael*

96 A toolkit enabling efficient, scalable and reproducible gene tagging in trypanosomatids

***Wheeler, Richard**; Dean, Samuel; Sunter, Jack D.; Hodkinson, Ian; Gluenz, Eva; Gull, Keith*

97 TARGET-BASED DISCOVERY OF NOVEL PAULLONES WITH ANTI-LEISHMANIAL ACTIVITY

***Medeiros, Andrea**; Korn, Ricarda; Benítez, Diego; Orban, Oliver; C. Ferreira, Vinicius; I. de Oliveira, Camila; Kunick, Conrad; Comini, Marcelo. A*

98 Post- translational regulation of Leishmania aquaglyceroporin AQP1

***Sharma, Mansi**; Mandal, Goutam; Bhattacharjee, Hiranmoy; Mukhopadhyay, Rita*

99 Cargo selection in the early secretory pathway of Trypanosoma brucei

***Kruzel, Emilia K.**; Zimmett III, George; Lowe, Tiffany; Bangs, James*

100 A glycosylation mutant of Trypanosoma brucei links social motility defects in vitro to impaired colonisation of tsetse in vivo

***Roditi, Isabel**; Imhof, Simon; Vu, Xuan Lan; Bütikofer, Peter*

101 Functional characterization of amino acid transporters in T. brucei

***Pereira de Macedo, Juan**; Mathieu, Christoph; Hürlimann, Daniel; Gonzalez, Amaia; Wirdnam, Corina; Suter Grotemeyer, Marianne; Zilberstein, Dan; Bütikofer, Peter; Rentsch, Doris*

102 Ectopic VSG overexpression: A tool to identify new players in the regulatory network of the expression site.

***Zimmermann, Henriette**; Batram, Christopher; Subota, Ines; Jones, Nicola; Engstler, Markus*

103 Characterisation of the interaction between the haptoglobin-haemoglobin receptor and its ligands in the context of the trypanosome cell surface.

MacGregor, Paula; Lane-Serff, Harriet; Higgins, Matthew; Carrington, Mark

104 Ultrastructural defects in Leishmania mexicana KHARON1 null mutant amastigotes

***Valli, Jessica**; Tran, Khoa; Vieira, Danielle; Gluenz, Eva; Landfear, Scott*

105 Investigating the complexity and dynamics of the minicircle repertoire in *Trypanosoma brucei*

***Schnaufer, Achim**; Cooper, Sinclair; Savill, Nicholas*

106 The unconventional nuclear envelope of kinetoplastid flagellates

***Koreny, Ludek**; Holden, Jennifer M.; Maishman, Luke; Obado, Samson; Rout, Michael P.; Field, Mark C.*

107 *Trypanosoma brucei* RNA editing ligase 1 (REL1) is involved in the religation of deletion, insertion, and non-canonical editing sites *in vivo*

***Schnaufer, Achim**; Jeacock, Laura; Ivens, Alistair*

108 Integrating a new screening strategy and insights from crystallography for drug discovery against Trypanosoma brucei.

***Campbell, Robert K.**; Pollastri, Michael; de Koning, Harry; Page, Rebecca; Bland, Nicholas; Olego, Sofia; Jadhav, Gopal; Colmenarejo-Sanchez, Gonzalo; de la Torre, Juana; Fouchet, Marie-Hélène; Berlanga de Lorenzo, Manuela; Martin, Julio; Manzano-Chinchon, Pilar*

109 Evolution of membrane trafficking in kinetoplastids

***Venkatesh, Divya**; J. O'Reilly, Amanda; Kelly, Steve; C. Field, Mark*

110 The mRNA-Bound Proteome in Trypanosoma brucei: towards a comprehensive regulatory network

***Erben, Esteban D.**; Lueong, Smiths; Hoheisel, Jorg; Clayton, Christine*

111 The nuclear protein UMSBP interacts with its mitochondrial paralogue and is involved in kinetoplast DNA replication and telomeric binding.

***Klebanov-Akopyan, Olga**; Glousker, Galina; Tzfati, Yehuda; Shlomai, Joseph*

112 Regulation of kDNA Replication Initiation by Post Translation Modifications

***Kapach, Guy**; Sela, Dotan; Shlomai, Joseph*

113 Is Vitamin C Biosynthesis essential in Trypanosoma cruzi?

***Taylor, Martin C.**; Logan-Klumpler, Flora; Fortes Francisco, Amanda; Lewis, Michael; Jayawardhana, Shiromani; Kelly, John*

114 ARM58, AN ANTIMONY RESISTANCE MARKER IN LEISHMANIA SPP

***Tejera Nevado, Paloma**; Schäfer, Carola; Nühs, Andrea; Clos, Joachim*

115 Ordering components of the slender to stumpy signalling pathway in Trypanosoma brucei

***McDonald, Lindsay**; Matthews, Keith*

116 TAC102 a novel component of the Tripartite Attachment Complex (TAC)

***Trikin, Roman**; Ochsenreiter, Torsten; Doiron, Nicolas; Schneider, Andre; Schnaufer, Achim; Schimanski, Bernd; Zuber, Benoit*

117 The kinetics of folate and antifolate drug transport in Trypanosoma brucei

***Dewar, Simon**; Ong, Han; Fairlamb, Alan*

118 Ion channels in Trypanosoma brucei as potential drug targets

***Steinmann, Michael**; Gonzàlez-Salgado, Amaia; Mäser, Pascal; Bütikofer, Peter; Sigel, Erwin*

119 VSG allelic exclusion through transcript interference in African trypanosomes

***Hutchinson, Sebastian**; Glover, Lucy; Trenaman, Anna; Wright, Jane; Horn, David*

120 A complete molecular toolkit for BioID proteomics and the identification of protein-protein interactions in vivo in trypanosomes

***Gould, Matt**; Boshart, Michael*

121 Identification of a novel Transcriptional Activator Factor that positively regulates VSG transcription in bloodstream trypanosomes

***Saura, Andreu**; López Farfán, Diana; Iribarren, Paula; Alvarez, Vanina; Navarro, Miguel*

122 TbPNT1 is essential for cell viability and kinetoplast maintenance in Trypanosoma brucei

***Grewal, Jaspreet Singh**; Mcluskey, Karen; Schnaufer, Achim; Das, Debanu; Mottram, Jeremy*

123 TriTrypDB: The Functional Genomics Resource for Kinetoplastids

***Harb, Omar**; Hertz-Fowler, Christiane; Roos, David; Silva, Fatima*

POSTERS: Session B
Monday 7:00pm

125 In vivo RITseq screening for drug targets in the Trypanosoma brucei kinome

***Fernandez-Cortes, Fernando**; Serafin, Tiago D.; Wilkes, Jonathan; Jones, Nathaniel G.; Ritchie, Ryan; McCulloch, Richard; Mottram, Jeremy C.*

126 The dihydroxyacetonephosphate acyltransferase TbDAT is essential for normal growth and synthesis of ether glycerolipids in Trypanosoma brucei procyclic forms

***Zufferey, Rachel**; Pirani, Karim; Dahlstrom, Kelly; Cheung-See-Kit, Melanie; Seerattan, Elizabeth; Williams, Tyler*

127 A novel PRMT heteromer in *Trypanosoma brucei*

***Kafkova, Lucie**; Fisk, John; Lott, Kaylen; Zhu, Lu; Read, Laurie*

128 A functional domain analysis of TbCC2D

***Shen, Qian**; Zhou, Qing; He, Cynthia*

129 TimX, a novel player in protein import across the inner mitochondrial membrane of T. brucei

***Harsman, Anke**; Oeljeklaus, Silke; Warscheid, Bettina; Schneider, André*

130 Genome-wide and protein kinase-focused RNAi screens reveal novel pathways of genome repair in Trypanosoma brucei

***McCulloch, Richard**; Serafim, Tiago; Black, Jennifer; Wilkes, Jonathan; Alsford, Samuel; Fernandez-Cortes, Fernando; Hamilton, Graham; Horn, David; Mottram, Jeremy*

131 An RNAi library screen reveals distinct parasite factors involved in the intracellular trafficking of apolipoprotein L1, the trypanolytic factor in human serum.

***Currier, Rachel**; MacLeod, Annette; Alsford, Sam*

132 Characterization of novel components involved in host adaptation in Trypanosoma brucei

***Cicova, Zdenka**; Dejung, Mario; Ritz, Sandra; Butter, Falk; Janzen, Christian*

133 Characterization of a novel telomere-binding protein in Trypanosoma brucei

***Reis, Helena**; Dejung, Mario; Kremmer, Elisabeth; Butter, Falk; Janzen, Christian J.*

134 A leucine aminopeptidase is involved in kinetoplast segregation in PCF Trypanosoma brucei

***Lukes, Julius**; Pena-Diaz, Priscila; Resl, Christian; Flegontov, Pavel; Flegontova, Olga*

135 Condensin depletion increases in situ VSG switching frequency in Trypanosoma brucei

***Rojas-Barros, Domingo**; Bart, Jean-Mathieu; Navarro, Miguel*

136 TbGem1 is required for normal mitochondrial morphology

***Niemann, Moritz**; Glauser, Melanie; Vock, Sévérine; Oeljeklaus, Silke; Warscheid, Bettina; Schneider, André*

137 Kinetics and routes of Trypanosoma brucei infection in the tsetse fly midgut

Cren, Christelle; Schuster, Sarah; Perrot, Sylvie; Bastin, Philippe; Rotureau, Brice

138 The cyclical development of Trypanosoma vivax in the tsetse fly

***Ooi, Cher Pheng**; Rotureau, Brice; Schuster, Sarah; Perrot, Sylvie; Bertiaux, Eloïse; Cren-Travaillé, Christelle; Cosson, Alain; Goyard, Sophie; Minoprio, Paola; Bastin, Philippe*

139 cAMP-mediated cell invasion by Trypanosoma cruzi

***Edreira, Martin M.**; Musikant, Daniel; Ferri, Gabriel; Mild, Jesica; Durante, Ignacio; Buscaglia, Carlos; Altschuler, Daniel*

140 Citrate metabolism in trypanosomes

***Ziebart, Nicole Emmy**; Allmann, Stefan; Huber, Claudia; Cahoreau, Edern; Portais, Jean Charles; Eisenreich, Wolfgang; Boshart, Michael*

141 Functional and Biochemical Analysis of the Trypanosoma brucei GPR89 Homologue as a Candidate Receptor for Environmental Cues

***Milne, Rachel M.**; Rojas, Federico; Thompson, Joanne; Matthews, Keith*

142 Highly unorthodox chromosome duplication revealed by mapping origins of DNA replication in Leishmania

***Marques, Catarina A.**; Dickens, Nicholas J.; Paape, Daniel; McCulloch, Richard*

143 In vitro and in vivo leishmanicidal activity of a cyclopalladated compound against Leishmania amazonensis and its possible inhibitory effect on recombinant Leishmania topoisomerase 1B

***Graminha, Marcia A.S.**; Velasquez, Angela M. A.; Ribeiro, Willian C.; Santoro, Mariana; de Souza, Rodrigo A.; Mauro, Antonio E.; Colepicolo, Pio; Desideri, Alessandro*

144 Development of Bioluminescent Trypanosoma cruzi Parasites expressing NanoLuc luciferase to study Chagas Disease Pathogenesis.

Silberstein, Erica M.

145 Using a mouse trypanosome to investigate immunomodulatory parasitic nematode gene function in vivo

***Vaux, Rachel**; Schnoeller, Corinna; Selkirk, Murray*

146 Do different African trypanosome species share quorum-sensing signal responses?

***Silvester, Eleanor**; Ivens, Alasdair; Matthews, Keith*

147 The cdc2-related kinase 9 (CRK9) forms a unique tripartite complex with a newly identified, L-type cyclin and a kinetoplastid-specific protein

***Badjatia, Nitika**; Park, Sung Hee; Ambrósio, Daniela; Kirkham, Justin; Günzl, Arthur*

148 Mutagenesis of the VSG 3'UTR identifies conserved sequences important for high level expression of VSG in bloodstream form trypanosomes

***Ridewood, Sophie L.**; Trenaman, Anna; Hall, Belinda S.; Ooi, Cher-Pheng; Rudenko, Gloria*

149 Genomic context and VSG expression

Ooi, Cher-Pheng; Ridewood, Sophie L.; Hall, Belinda S.; Trenaman, Anna; Rudenko, Gloria

150 Genome localization of RNA polymerases I and II by ChIP-seq analyses in bloodstream form trypanosomes

***Cordon-Obras, Carlos**; Navarro, Miguel; Lopez-Farfan, Diana; Bart, Jean Mathieu; Lorenzo, Fabian; Valladares, Basilio; Carrington, Mark; Dickens, Nicholas J.*

151 Towards new drugs for trypanosomatid diseases based on specific high-affinity inhibitors for Trypanosoma brucei kinetoplastid RNA editing ligase 1

***Zimmermann, Stephan**; Feher, Victoria; Sorensen, Jesper; Smith, Chris; Hall, Laurence; Greaney, Michael; Amaro, Rommie; Schnaufer, Achim*

152 Trypanosoma brucei sphingomyelinase enzymes as targets for drug development

Dickie, Emily

153 Translesion DNA Polymerases and genome maintenance in Trypanosoma brucei

***Zurita, Andrea**; Prorocic, Marko; McCulloch, Richard*

154 A new target for a novel class of nitro-heterocyclic-amides with potent trypanocidal activity

***SMITH, TERRY K.**; Trouche, Nathalie; Ciapetti, Paola*

155 Dissecting the kinome of T. brucei: RIT-seq of cell cycle sorted T. brucei identifies kinases involved in the regulation of nuclear DNA replication

***Paape, Daniel**; Marques, Catarina A.; Wilkes, Jonathan; Fernandez-Cortes, Fernando; Serafim, Tiago D.; Mottram, Jeremy C.; McCulloch, Richard*

156 Elucidating the target and structure-activity relationships of novel natural product inspired Trypanosoma brucei inhibitors

***King, Elizabeth**; Fraser, Andrew; Gould, Eoin; Menzies, Stefanie; Tulloch, Lindsay; Zacharova, Marija; Smith, Terry; Florence, Gordon*

157 Assessing Inosine Monophosphate Dehydrogenase as a drug target in Trypanosoma brucei

***Ong, Han**; Wyllie, Susan; Fairlamb, Alan*

158 Synchronous expression of individual metacyclic VSG genes in Trypanosoma brucei and CITFA binding to metacyclic expression site promoters

***Kolev, Nikolay G.**; Ramey-Butler, Kiantra; Ullu, Elisabetta; Günzl, Arthur; Tschudi, Christian*

159 Cleavage specificity of the editosome endonucleases in vivo

***Carnes, Jason**; McDermott, Suzanne; Gould, Matthew; Schnaufer, Achim; Stuart, Ken*

160 Investigating the Essential Function of kDNA Polymerase IC in Trypanosoma brucei.

***Miller, Jonathan**; Concepción, Jeniffer; Klingbeil, Michele*

161 Glucose-dependent dual localization of the glycosome protein TbPEX13.1
***Bauer, Sarah**; Morris, Meredith*
162 TbPif1, A mitochondrial helicase with dynamic localization during cell cycle
***Rocha-Granados, Maria C.**; Springer, Amy; Klingbeil, Michele*
163 Spatiotemporal localization of the mitochondrial DNA Polymerase IB in Trypanosoma brucei
***Rivera, Sylvia**; Klingbeil, Michele; Concepcion-Acevedo, Jeniffer; Greene, Rebbeca*
164 Characterization of an Actin-Binding Protein (profilin) in Trypanosoma cruzi
***Osorio Mendez, Juan Felipe**; Manning, Rebeca; Hernandez, Roberto; Cevallos, Ana Maria*
165 Knockdown of IC138 in Trypanosoma brucei: a motility mutant with minor indirect effects.
***Springer, Amy L.**; Greene, Rebecca; Wilson, Corinne S.*
166 Towards a protein complex map of Trypanosoma brucei
***Mehta, Vaibhav N.**; Gazestani, Vahid; Nikpour, Najmeh; Najafabadi, Hamed; Moshiri, Houtan; Salavati, Reza*
167 Down regulation of Tim50 in Trypanosoma brucei increases tolerance to oxidative stress
***Chaudhuri, Minu**; Singha, Ujjal; Fullerton, Marlorie*
168 Does the DNA sequence affect nucleosome positioning in T. brucei?
***Wedel, Carolin**; Förstner, Konrad U.; Siegel, T. Nicolai*
169 Control of multivesicular body function by PI(3,5)P2 in Trypanosoma brucei
***Gilden, Julia K.**; Mansfield, John; Bangs, James*
170 Post-transcriptional regulation of Leishmania aquaglyceroporin AQP1
***Mandal, Goutam**; Mandal, Srotoswati; Sharma, Mansi; Charret, Karen Santos; Papadopoulou, Barbara; Bhattacharjee, Hiranmoy; Mukhopadhyay, Rita*
171 Characterization of actin 2, an actin variant of Trypanosoma cruzi.
***Vizcaíno-Castillo, Andrea**; Roberto, Hernandez; Cevallos, Ana Maria*
172 Thioredoxin-monothiol glutaredoxin hybrid protein from Trypanosoma brucei: biochemistry, structure and biological relevance
***Bonilla, Mariana M.**; Manta, Bruno; Bellanda, Mássimo; Comini, Marcelo*
173 Translational repression by the 3' UTR of the TbZC3H11 mRNA
***Minia, Igor**; Clayton, Christine*
174 Distinct phenotypes caused by mutation of MSH2 in trypanosome insect and mammalian life cycle forms are associated with parasite adaptation to cope with oxidative stress
Grazielle-Silva, Viviane; Zeb, Tehseen; Bolderson, Jason; Campos, Priscila; Miranda, Julia; Alves, Ceres; Machado, Carlos Renato; McCulloch, Richard; Teixeira, Santuza

175 The early-branching trypanosomatid Paratrypanosoma confusum has a complex life cycle

***Dobakova, Eva**; Lukes, Julius; Skalicky, Tomas; Flegontov, Pavel; Tesarova, Martina; Jirsova, Dagmar; Votypka, Jan; Yurchenko, Vyacheslav*

176 Trypanosoma brucei RAP1 plays an important role in suppressing subtelomeric VSG associated gene conversion by reducing R-loops at telomeres

***Nanavaty, Vishal P.**; Pandya, Unnati; Li, Bibo*

177 Targeting Host Cell Kinases As Therapy for Leishmaniasis

***Wetzel, Dawn M.**; Rhodes, Emma L.; Li, Shaoguang; McMahon-Pratt, Diane; Koleske, Anthony J.*

POSTERS: Session C
Tuesday 7:00pm

179 A Murine Model of Trypanosome Lytic Factor 2

***Verdi, Joseph**; Zipkin, Ron; Raper, Jayne*

180 You aren't what you don't eat: metabolic specialization is a major driver of genome evolution in kinetoplastids

***Seward, Emily**; Kelly, Steve*

181 Cyclic AMP binding of a novel signal transducer (CARP1) in T. brucei

***Polatoglou, Eleni**; Omelianczyk, Radoslaw; Brennand, Ana; Bachmaier, Sabine; Schwede, Frank; Genieser, Hans-Gottfried; de Koning, Harry; Boshart, Michael*

182 Quantified markers for the Leishmania lifecycle progression to infectious forms

***De Pablos, Luis Miguel**; Walrad, Pegine*

183 Investigating Leishmania development in human: from system analysis to molecular mechanism

***Zilberstein, Dan**; Myler, Peter, J.; Tsigankov, Polina*

184 TbTIF2 and TbTRF suppress VSG switching through common and independent mechanisms

***Jehi, Sanaa**; Li, Bibo*

185 Investigating Machine Learning Methods to Characterize Origins of DNA Replication in Kinetoplastid Genomes

***Campbell, Samantha**; Marques, Catarina; McCulloch, Richard; Dickens, Nicholas*

186 New approach to chemotherapy: Drug-induced differentiation leads to lysis of African trypanosomes

***Wenzler, Tanja**; Schumann Burkard, Gabriela; Mäser, Pascal; Roditi, Isabel; Brun, Reto*

187 Genetic interaction between base J and other chromatin factors

***Kim, Hee-Sook**; Schulz, Danae; Cross, George; Papavasiliou, Nina*

188 Sphingolipid biosynthesis in kinetoplastids.

***Ciganda, Martin**; Bangs, Jay*

189 The putative CorA homolog of Trypanosoma brucei is not a magnesium transporter

***Schmidt, Remo S.**; Greganova, Eva; Steinmann, Michael E; Wirdnam, Corina; Rentsch, Doris; Sigel, Erwin; Mäser, Pascal*

190 An Investigation into the (2-aminoethyl)phosphonate Pathway in Trypanosoma cruzi

***Coron, Ross P.**; Smith, Terry K.*

191 Functions of MRB1 complex proteins in kinetoplastid RNA editing illuminated by deep sequencing of partially edited RNA populations

***Simpson, Rachel**; Bruno, Andrew; Sun, Yijun; Read, Laurie*

192 Too much of a good thing? The curious case of Leishmania sphingosine kinase

***Zhang, Kai**; Balyimez, Aysegul; Zhang, Ou*

193 The flagellar lipidome of trypanosomes

***Sharma, Aabha**; Gazos-Lopes, Felipe; Tyler, Kevin; Almeida, Igor; Engman, David*

194 Functions of pentatricopeptide repeat (PPR) RNA binding proteins in mitochondrial mRNA polyadenylation

***Afasizheva, Inna**; Zhang, Liye; Monti, Stefano; Afasizhev, Ruslan*

195 Acidocalcisome-mediated autophagy in Trypanosoma brucei

***Li, Feng-Jun**; He, Cynthia Y.*

196 The catalase – an enzyme selectively present only in monoxenous trypanosomatids

***Horáková, Eva**; Kraeva, Natalya; Faktorová, Drahomíra; Korený, Ludek; Yurchenko, Vyacheslav; Lukeš, Julius*

197 FPC4: a special guest at the FPC party

***Albisetti, Anna**; Florimond, Célia; Landrein, Nicolas; Eggenspieler, Marie; Dacheux, Denis; Robinson, Derrick R; Bonhivers, Mélanie*

198 Deep proteomic analysis of T. brucei FoF1-ATP synthase reveals unique features

***Gahura, Ondrej**; Vachova, Hanka; Subrtova, Karolina; Panicucci, Brian; Walker, John E.; Zikova, Alena*

199 Functional Analysis of a Novel and Essential Subunit of FoF1-ATP synthase in T. brucei

***Subrtova, Karolina**; Panicucci, Brian; Zikova, Alena*

200 Phenotypic and transcriptomic changes during Leishmania donovani culture adaptation inform on potential mechanisms of virulence attenuation.

***Pescher, Pascale**; Guerfali, Fatma; Friedman, Robin; Dilliès, Marie-Agnès; Proux, Caroline; Kedra, Darek; Prieto-Barja, Pablo; Schwikowski, Benno; Coppée, Jean-Yves; Notredame, Cédric; Späth, Gerald*

201 Characterising the trypanosomatid lysosome and its essential role in host lipid catabolism

***Young, Simon A.**; Hacker, Christian; Lucocq, John; Smith, Terry K.*

202 The proteome of T. brucei nuclear periphery granules

***Goos, Carina**; Meyer-Natus, Elisabeth; Dejung, Mario; Stigloher, Christian; Engstler, Markus; Butter, Falk; Kramer, Susanne*

203 Tandem affinity purification of trypanosomal DNA polymerase delta identifies a novel essential subunit

***Cowton, Andrew**; Smith, Terry; MacNeill, Stuart*

204 Understanding Novel Functional Properties and Interactions Associated with a Leishmania Poly-A Binding Protein Homologue (PABP1)
***de Melo Neto, Osvaldo P.**; Xavier, Camila; da Costa Lima, Tamara; Merlo, Kleison; Reis, Christian; Papadopoulou, Barbara*

205 CHARACTERIZATION OF NEW eIF4F SUBUNITS IN Trypanosoma brucei
***Freire, Eden**; Vashisht, Ajay; Malvezzi, Amaranta; Wohlschlegel, James; de melo Neto, Osvaldo; Sturm, Nancy; Campbell, David*

206 Tim62, a Novel Mitochondrial Protein in Trypanosoma brucei, is Essential for Stability and Assembly of the TbTim17 Protein Complex
***SINGHA, UJJAL K.**; Hamilton, VaNae; Chaudhuri, Minu*

207 Identification and functional characterization of a Leishmania major HSP70-related protein with an unusual domain structure
***Drini, Sima**; Rachidi, Najma; Späth, Gerald*

208 Stalled ribosomes and the control of translation in the infective metacyclic trypomastigote forms of Trypanosoma cruzi.
***Dallagiovanna, Bruno**; Bispo, Saloe; Holetz, Fabiola; Belew, Ashton Trey; Guerra-Slompo, Eloise*

209 The regulation of the mRNA expression by PUF3 could be a mechanism to explain the resistance to benznidazole in trypanosomes
***Triana, Omar**; Mejia-Jaramillo, Ana Maria; Díez-Mejía, Andres Felipe; Echeverri-Gaitán, María Clara; Kelly, John*

210 Characterisation of Leishmania casein kinase 1 isoform 2 localization and interaction
***Martel, Daniel**; Najma, Rachidi; Pine, Stewart; Bartsch, Katerina; Clos, Joachim; Spaeth, Gerald*

211 In search of glycosomal nucleotide sugar transporters
***Guther, Lucia**; Wu, Di; Ferguson, Michael*

212 Functional Significance of the Evolution and Architecture of the Trypanosome Nuclear Pore Complex
***Obado, Samson**; Brillantes, Marc; Zhang, Wenzhu; Ketaren, Natalia; Uryu, Kunihiro; Field, Mark; Chait, Brian; Rout, Michael*

213 The dynamics of VSG coat replacement during antigenic variation in African trypanosomes
***Pinger, Jason**; Hovel-Miner, Galadriel; Papavasiliou, Nina*

214 Energy metabolism in different Trypanosoma cruzi lifecycle stages and the impact of host metabolism on intracellular amastigotes.
***Shah-Simpson, Sheena**; Caradonna, Kacey; Burleigh, Barbara*

215 An RNAi toolkit for functional genetic analysis of Leishmania (Viannia) braziliensis

***Lye, Lon-Fye**; Owens, Katherine; Brettman, Erin; Jang, Soojin; Akopyants, Natalia S.; Beverley, Stephen M.*

216 A new software resource for rapid automatic annotation of kinetoplastid genomes

***Steinbiss, Sascha**; Silva-Franco, Fatima; Brunk, Brian; Otto, Thomas Dan; Ramasamy, Gowthaman; Myler, Peter; Roos, David; Beverley, Stephen; Warren, Wes; Hertz-Fowler, Christiane; Berriman, Matt*

217 Decoding anti-leishmanial drug efficacy and resistance mechanisms using RNAi library screening in Trypanosoma brucei.

***Alsford, Sam**; Baker, Nicola; Hutchinson, Sebastian; Horn, David*

218 A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in Leishmania donovani

***Martin, Jessica L.**; Fulwiler, Audrey; Yates, Philip A.; Boitz, Jan M.; Ullman, Buddy; Carter, Nicola S.*

219 A Zinc finger-containing RNA binding protein impacts mitochondrial gene expression in Trypanosoma brucei

***Ammerman, Michelle**; Volobuev, Denis; Downey, Kurtis; Read, Laurie*

220 Identifying transcription termination sites in trypanosoma brucei

***Lopes da Rosa-Spiegler, Jessica**; Sabatini, Robert*

221 Characterization and DNA Sequence Specificity of the Base J Associated Glucosyltransferase

***Bullard, Whitney**; Cliffe, Laura; Wang, Pengcheng; Wang, Yinsheng; Sabatini, Robert*

222 Development of biological assays to assist Human African Trypanosomiasis (HAT) drug discovery

***Rao, Srinivasa P S**; Vanessa, Manoharan; Thayalan, Pamela; Koh, Hazel*

223 PATHOGEN MEDIATED EVOLUTION OF APOL1 HAPLOTYPES

Pant, Jyoti

224 Evolution of Protein Palmitoyl Acyltransferases in Kinetoplastids

***Brown, Robert W.**; Goldston, Amanda; O'Reilly, Amanda; Emmer, Brian; Field, Mark; Engman, David*

225 Non-Mitochondrial mRNA Uridylation in T.brucei

***Knusel, Sebastian**; Zhang, Liye; Roditi, Isabel; Aphasizhev, Ruslan*

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

***DeMarco, Stephanie**; Hill, Kent*

227 Elucidating the Mechanism of TLF Mediated Trypanosome Killing ActivityAbdurakhmanov, Izrail*; Raper, Jayne*

228 High throughput screening assay to identify novel inhibitors of Trypanosoma brucei from a collection of botanical extracts and small molecules

Stubblefield, Jeannie M.*; Gross, Alexis; Wright, Matthew; Pathiranage, Anuradha; Handy, Scott; Newsome, Anthony*

229 Cis and Trans Effects of a Regulatory RNA Helicase on Substrate Loading and Maturation in the RNA Editing Apparatus in Kinetoplastids.

MADINA, BHASKARA REDDY*; KUMAR, VIKAS; MOOERS, BLAINE; BUNDSCHUH, RALF; CRUZ-REYES, JORGE*

230 Substrate-bound RNA helicase and Scaffold RNPs Control RNA Editing in kinetoplastids

Kumar, Vikas*; Madina, Bhaskar; Mooers, Blaine; Read, Laurie*

231 TWO RELATED TRYPANOSOMATID eIF4G HOMOLOGUES HAVE FUNCTIONAL DIFFERENCES COMPATIBLE WITH DISTINCT ROLES DURING TRANSLATION INITIATION

Moura, Danielle*; Reis, Christian; Xavier, Camila; da Costa Lima, Tamara; Lima, Rodrigo; Carrington, Mark; de Melo Neto, Osvaldo*

SESSION I: Gene Expression

Robert Sabatini, Chair.

Saturday 7:00pm

04/25/2015

1A The Inositol Phosphate Pathway Controls Transcription of Telomeric Expression Sites in Trypanosomes

Cestari, Igor (Seattle BioMed)*; Stuart, Ken (Seattle BioMed)*

African trypanosomes transcribe only one of numerous telomeric expression sites (ESs) with its VSG gene and undergo antigenic variation by switching transcription between ESs or by recombination. We show that steps in the inositol phosphate (IP) pathway specifically affects transcription of ESs in bloodstream form Trypanosoma brucei . Transcription knockdown of phosphatidylinositol 5-kinase (TbPIP5K), phosphatidylinositol 5-phosphatase (TbPIP5Pase), or overexpression of phospholipase C (TbPLC) each derepress multiple silent ESs. Monoallelic expression is restored after temporary knockdown of TbPIP5K but results in frequent switching of the VSG gene expressed. Transcription of multiple ESs correlates with increased numbers of telomeric and RNA polymerase I foci which colocalize at multiple extranucleolar sites. TbPIP5K, TbPLC, their substrates and products localize to the plasma membrane. However, TbPIP5Pase is nuclear, proximal to telomeres and is associated with the repressor/activator protein 1 (TbRAP1) and their associations and silencing function are altered by knockdown of TbPIP5K. These results show that specific steps in the IP pathway control the monallelic transcription of ESs as well as antigenic switching in T. brucei by epigenetic regulation of telomere silencing.

1B Allelic exclusion by VEX1 controls antigenic variation in trypanosomes

Glover, Lucy (University of Dundee)*; Hutchinson, Sebastian (University of Dundee); Horn, David (University of Dundee)*

Allelic exclusion coordinates expression among gene-families, underpinning antigenic variation in parasites and olfaction in mammals. Although suspected, no protein regulator has previously been identified that specifically associates with the single active allele. One paradigm for antigenic variation is Variant surface glycoprotein (VSG) switching in the bloodstream African trypanosome. Multiple VSG genes found adjacent to telomeres are competent for expression, but only a single subtelomeric VSG is transcribed and millions of molecules of this expressed VSG form a dense coat on each cell. The active VSG is associated with a concentrated focus of RNA polymerase-I at an extranucleolar site known as the expression-site body (ESB). Here, we describe a putative SWIM-type zinc-finger protein required for VSG allelic exclusion. VEX1 (VSG EXclusion 1, Tb927.11.16920) was identified using an RNA interference library screen for loss of telomeric reporter-gene silencing in bloodstream-form trypanosomes. VEX1 depletion also lead to loss of VSG silencing, resulting in cells coated with multiple VSGs. VEX1 accumulates at a single ESB-associated focus that persists through the cell-cycle. We find that a synthetic VSG reporter cassette and the native VSG display VEX1-dependent exclusion. Additional synthetic RNA polymerase-I reporter cassettes also display VEX1-dependent exclusion but only when they share common sequences. Thus, VEX1 is a VSG monoallelic regulator that restricts RNA polymerase-I transcription to a single subtelomere at a single extranucleolar focus. This transcription negatively regulates related transcription units in trans and underpins antigenic variation in the African trypanosome.

1C An RNA-binding protein controls VSG gene silencing in African Trypanosomes

Trenaman, Anna (University of Dundee)*; Glover, Lucy (University of Dundee); Hutchinson, Sebastian (University of Dundee); Horn, David (University of Dundee)*

The expression of a single Variant Surface Glycoprotein (VSG) from one among many telomeric Expression Sites (ESs) underlies antigenic variation in African trypanosomes. The mechanisms controlling this monotelomeric ES transcription and concurrent silencing of the remaining ESs remain to be fully elucidated. We ran an RNAi screen in bloodstream form T. brucei that implicated RNA-binding proteins in gene-silencing. One of these proteins, known as ZC3H40, was found to play a role in VSG-silencing. A ZC3H40 orthologue known as CSBPB in Crithidia fasiculata was previously shown to form an RNA-binding complex with CSBPA, equivalent to ZC3H39 in T. brucei (PMID: 11416125 / 12730192) and both proteins were recently implicated in stimulating gene expression in a high-throughput mRNA-tethering screen (PMID: 24945722). We found that depletion of either protein disrupted VSG ES silencing as determined by western blotting, flow-cytometry and RNA-seq. The presence of a ZC3H39/40 complex in T. brucei was supported by immunofluorescence analysis, depletion of both proteins when only one was targeted by RNAi and pull-down experiments. Gene knockouts have recently been generated for further analysis. Thus, we report the first known RNA-binding proteins that impact monotelomeric VSG expression. We are currently seeking to identify the transcripts that bind the ZC3H39/40 complex.

1D Regulation of RNA Pol II transcription termination by H3V and base J modified DNA

Reynolds, David (University of Georgia)*; Siegel, Nicolai; Beverley, Stephen; Sabatini, Robert*

The modified DNA base J, ß-D-glucosyl-hydroxymethyluracil, is enriched at RNA Polymerase (Pol) II transcription initiation and termination sites, TSSs and TTSs, respectively, in kinetoplastids. J functions in the regulation of Pol II termination at TTSs within convergent strand switch regions (cSSRs) in Leishmania spp., preventing readthrough transcription and subsequent production of antisense RNAs. In Trypanosoma brucei , base J and histone H3 variant (H3V) co-localize at TTS. We now show that removal of base J or H3V in T. brucei does not lead to readthrough transcription at cSSRs genome-wide, but does result in termination defects within gene clusters, increasing the expression of downstream genes. The combined loss of J and H3V results in further de-repression, indicating J and H3V independently regulate Pol II termination. In L. major we find H3V also co-localizes with J at TTSs and its removal reduces the level of J at these sites, implicating chromatin structure in the regulation of J synthesis. Surprisingly, despite J reduction, loss of H3V does not result in termination defects, indicating H3V does not regulate Pol II termination in L. major . Treating the H3V knockout cells with DMOG to inhibit J synthesis however, results in a further reduction of J at TTSs and a strong growth defect that is not observed in wild-type cells. This is accompanied by more severe termination defects and uncovering of defects at sites not previously detected in DMOG treated wild-type L. major . These results indicate kinetoplastids differentially utilize epigenetic modifications to regulate gene expression and to prevent the production of aberrant transcripts.

1E Global transcriptome profiling of the human macrophage infected with Leishmania major, Leishmania amazonensis and latex beads

El-Sayed, Najib M. (University of Maryland)*; Fernandes Dupecher, Cecilia (University of Maryland College Park); Dillon, Laura A.L. (University of Maryland College Park); Belew, A. Trey (University of Maryland College Park); Hughitt, V. Keith (University of Maryland College Park); Okrah, Kwame (University of Maryland College Park); Corrada Bravo, Hector (University of Maryland College Park)*

Until recently, most studies of Leishmania immunobiology and genetics, as well of host parasite interactions, have made use of murine models of infection to mimic human disease. In this study, we conducted a high resolution transcriptomic analysis of the global changes of gene expression in human macrophages infected with metacyclic forms of L. major (cuteaneous self-healing) and L. amazonensis (cutaneous self-healing/ cutaneous diffuse). In addition, we compared these results to the transcriptome profile of latex bead-loaded macrophages to be able to characterize the biological response of the macrophage towards the parasite, while taking into account the generic phagocytic response to inert particles. Simultaneously we interrogated the transcriptome of the parasites at different time points of interaction to obtain a comprehensive profile of the changes in the steady state RNA during the course of infection. Our preliminary results highlight a significant transcriptome remodeling of the infected human macrophage during the early time points of infection with either L. major or L. amazonensis when compared to cells at later periods. A relatively small subset of genes that are highly differentially expressed (>2x) emerges, however, when we exclude the phagocytic effect of inert particles in macrophages. Similarly, when comparing the macrophage's response to different Leishmania species that may have different clinical outcomes, we identified a few differentially expressed genes that may represent a species-specific response. The unprecedented transcriptomic profiling that we present here sheds light on the biological processes that occur during the interaction of human macrophages and different Leishmania species.

1F Dynein Light Chain DYNLL1/LC8 is Essential for RNA Polymerase I Transcription in Trypanosoma brucei

Kirkham, Justin (University of Connecticut Health Center)*; Park, Sung Hee (University of Connecticut Health Center); Nguyen, Tu (University of Connecticut Health Center); Gunzl, Arthur (University of Connecticut Health Center)*

In Trypanosoma brucei , ribosomal RNA and variant surface glycoprotein (VSG), both of which are essential for parasite viability, are expressed by RNA polymerase I (RNAPI) and require the promoter-binding class I transcription factor A (CITFA) complex. The specific roles of the eight individual CITFA subunits, however, remain unclear. Previous data revealed DYNLL 1 (LC8) as one of the CITFA subunits, and given the emerging view that this protein functions as a molecular hub with both localizing and regulatory functions, we began investigating the role of DYNLL1 in RNAPI transcription. DYNLL1 silencing clearly affected VSG mRNA expression, indicating that this subunit is important for RNAPI function. CITFA sedimentation profiles, co-immunoprecipitation of recombinant protein, and bioinformatic analysis revealed a DYNLL1 binding site near the N-terminus of the subunit CITFA2. To test the functional significance of this site, we introduced an RNAi-resistant CITFA2 transgene into cell lines in which the endogenous CITFA2 gene was conditionally silenced. These experiments demonstrated that the DYNLL1-CITFA2 interaction is essential for RNAPI transcription because CITFA2 with a mutated DYNLL1 binding site, in contrast to wild-type, did not rescue the lethal knockdown phenotype or the decline of RNAPI transcripts. Further investigation revealed that while mutant CITFA2 localized to the nucleus, it failed to bind to RNAPI promoters and failed to assemble into the CITFA complex. The importance of DYNLL1 for CITFA2 inclusion into the CITFA complex was confirmed using a DYNLL1 knockdown cell line, which demonstrated an increase in the amount of disassociated CITFA2 upon DYNLL1 knockdown. Together, these results show that, among other functions, DYNLL1 and its interaction with CITFA2 are essential for productive RNAPI transcription in Trypanosoma brucei.

1G A single-point mutation in the RNA-binding protein 6 enables progression through the complete life cycle of Trypanosoma brucei in vitro

Shi, Huafang (Yale School of Medicine)*; Kolev, Nikolay G. (Yale Univeristy Medical School); Ullu, Elisabetta (Yale Univeristy Medical School); Tschudi, Christian (Yale Univeristy Medical School)*

We previously established an in vitro differentiation system based on the overexpression of the RNA binding protein 6 (RBP6), which recapitulates Trypanosoma brucei differentiation in the tsetse vector. Thus far, overexpression of RBP6 induced differentiation of procyclics to infectious metacyclics (MFs) in culture, and further differentiation to bloodstream forms (BSFs) required infection of mice. However, we serendipitously isolated a single point mutation in RBP6 (Q109K), whose expression not only generated MFs, but induced further differentiation so that purified MFs continued the developmental cycle to BSFs expressing VSG-2, formerly known as VSG 221. The proportion of cells expressing the various metacyclic VSGs closely mirrored the induction of wild-type RBP6. By RT-PCR, VSG-2 was first detectable 2 days after the purified metacyclics were placed into BSF media and at 370C, and metacyclic VSG 397 remained noticeable up to 10 days. To begin to probe the early events triggered by RBP6, we surveyed the transcriptome in cells expressing wild-type and mutant RBP6 (Q109K) at 0, 2, 6, and 24 hours after induction. In addition, RNA samples were isolated from cells overexpressing the mutant RBP6 0, 1, 2, 4, 8 and 11 days after placement in bloodstream culture conditions and subjected to high-throughput sequencing. The results from both RNA-Seq will be presented. This system provides the first opportunity to study at the mechanistic level the switch from metacyclic VSG gene expression to bloodstream form-specific VSG genes.

1H Tracking the in vivo dynamics of antigenic variation in Trypanosoma brucei

Mugnier, Monica (Rockefeller University)*; Cross, George A. M. (Rockefeller University); Papavasiliou, F. Nina (Rockefeller University)*

Using a repertoire of over 2000 different variant surface glycoprotein (VSG) genes within its genome, Trypanosoma brucei changes its dense VSG surface coat to avoid detection by the immune system of its mammalian host. The dynamics of antigenic variation in T. brucei during an infection, however, are poorly understood. How many variants appear over the course of an infection? Is there a pattern to VSG expression over time? Although some of these questions have been broached using Sanger sequencing of VSG cDNA, technical limitations have prevented a high-resolution, quantitative study of VSG expression during T. brucei infection. We have developed a method, termed VSG-seq, for quantitatively examining the diversity of expressed VSGs in any population of trypanosomes. Based on de novo assembly, this approach can be used for the high-resolution study of VSG expression in any strain of T. brucei, whether in the lab or in the field. Using VSG-seq, we have studied the dynamics of VSG expression during pleiomorphic T.brucei infections, and our experiments reveal more dynamic and nuanced host-parasite interactions than previously expected. Nearly a quarter of the full-length VSG repertoire is sampled within only four peaks of infection, and this level of diversity persists throughout. We see as many as 83 different variants at a single time point and an average of nearly 30 per sampling. In addition, the appearance and disappearance of mosaic VSGs during infection hint at the mechanism by which these VSGs form. These results raise many new questions about the T. brucei host-pathogen interaction, and VSG-seq allows access to this interface in its proper context: the natural host.

SESSION II: Cell Biology I

Philippe Bastin, Chair.

Sunday 8:45am

04/26/2015

2A The kinetoplastid parasite flagellum: the current state of our ignorance.

Gull, Keith (University of Oxford)

Shape and form in kinetoplastid parasites is defined, in the main, by an internal microtubule based cytoskeleton. However, a defining feature of these organisms is the existence of a flagellum whose nature varies somewhat between cell types. Epimastigotes, trypomastigotes and amastigotes are distinguished by the substructure of their flagella, the length of attachment to the cell body and other functional elaborations. In turn, the basal body complex – defining a "master organiser" region of the cell – orchestrates a variety of cellular microtubules and filament systems essential for inheritance of cell shape and completion of cytokinesis, in addition to regulating kinetoplast position and segregation. Critically, it also defines perhaps one of the most important pathogenicity attributes – the flagellar pocket. Much elegant work in many laboratories has contributed to an understanding that now maps some (most?) of the components of the flagellum to general positions with insights to function. Evolutionary cell biology and comparisons with other model organisms highlights conserved features and specialisations of kinetoplastid flagella. All this has enabled a corpus of knowledge providing fascinating insights to a complex organelle involved in the biology of movement, shape, sensing, proliferation and pathogenicity. This progress has also highlighted the current state of our ignorance – including the knows we only think we know, the known unknowns and the unknown unknowns.

2B Late steps in exocytosis: Expansions, losses and novel trypanosome subunits for the exocyst

Boehm, Cordula (University of Dundee, Division of Biological Chemistry and Drug Discovery)*; Obado, Samson (The Rockefeller University, New York); O'Reilly, Amanda (University of Dundee, Division of Biological Chemistry and Drug Discovery); Gadelha, Catarina (University of Nottingham, School of Life Sciences); Chait, Brian (The Rockefeller University, New York); Rout, Michael (The Rockefeller University, New York); Field, Mark (University of Dundee, Division of Biological Chemistry and Drug Discovery)*

The evolution of the endomembrane system of eukaryotic cells has been reconstructed in some detail, with recognition that complexes containing paralogous members of large gene families are responsible for specifying organelle identity and controlling transport between compartments. However, evidence is emerging for lineage-specific modifications to these basic core systems, encompassing both gain of lineage-specific proteins and secondary loss. Using a combination of comparative genomics, cryoimmunoisolation, proteomics and imaging, we have investigated the evolution of the exocyst complex, a multimeric complex that targets post-Golgi vesicles to the plasma membrane. In animals and fungi the exocyst is an octameric complex that consist of two subcomplexes of three and five subunits each. By cryomilig and affinity capture of the exocyst from Trypanosoma brucei using TbSec15 as an affinity handle we have identified Exo99, a ninth exocyst subunit restricted to Kinetoplastida and Amoeabazoa. Exo99 is essential in bloodstream form trypanosomes; its depletion is rapidly lethal and leads to cells with a round morphology and an enlarged flagellar pocket reminiscent of the clathrin/Rab11 depletion phenotype. Furthermore we found that depletion of both Sec15 and Exo99 generates defects in cargo uptake and delivery to the lysosome suggesting a so far unknown function of exocyst components in endocytic events. Taken together these data provides evidence not only for lineage-specific evolutionary events at the plasma membrane, but also calls for a new view on exocyst function.

2C A novel AMPKalpha1-TOR4 pathway regulates differentiation from proliferative bloodstream to quiescent stumpy form in Trypanosoma brucei

Saldivia Concepción, Manuel Alejandro (Instituto de Parasitología y Biomedicina "López-Neyra" (IPBLN) Consejo Superior de Investigaciones Científicas (CSIC)); *Navarro, Miguel (Instituto de Parasitología y Biomedicina "López-Neyra" (IPBLN) Consejo Superior de Investigaciones Científicas (CSIC))*

The trypanosomatid life cycle proceeds through different stages including a quiescent stage pre-adapted to host transitions. TOR (target of rapamycin) is a kinase that controls cell growth in eukaryotes in response to nutrients, energy conditions, and growth factors. In addition to the classical TbTOR1 and TbTOR2 orthologs, another TOR kinase, named TbTOR4, negatively regulates the developmental differentiation process to the quiescent stumpy form in Trypanosoma brucei . AMP analogues and hydrolysable analogues of cAMP were found to inhibit TbTOR4 and induce the stumpy-like quiescent form (Barquilla et al., PNAS 2012). These results suggest that AMPK (AMP activated Kinase), a well-known upstream regulator of mTOR in eukaryotes, might be involved in this developmental regulation. To investigate this possibility we biochemically identified the trypanosome AMPK complexes by LC-MS/MS analysis. TbAMPKa1 (Tb927.10.5310) and TbAMKa2 (Tb927.3.4560) were found to form complexes with the conserved subunits TbAMPKß (Tb927.8.2450) and TbAMPK? (Tb927.10.3700). These complexes copurified with several proteins including TbBIP, TbGSK3 and metabolism regulators, which have been previously described as AMPK-associated proteins in other eukaryotes. Using in vitro phosphorylation assays, we show that TbAMPKa1 activity is induced by AMP analogues and inhibited by compound C, an AMPK kinase inhibitor. Importantly, we show that trypanosome AMPKa1 activation occurs in vivo during the differentiation of pleomorphic bloodstreams to the quiescent stumpy form, and this activation is concomitant with TOR4 inactivation. Conversely, in vivo differentiation from the proliferative to the stumpy bloodstream form is inhibited by compound C treatment of infected mice. We here provide evidence for a link between AMPKa1 activation and stumpy differentiation, suggesting that the AMPKa1-TOR4 signalling pathway is central to the regulation of this process

2D Towards a first molecular model of the Tripartite Attachment Complex (TAC) and its dynamics during the cell cycle in Trypanosoma brucei

Hoffmann, Anneliese (University of Bern)*; Doiron, Nicholas (University of Bern); Trikin, Roman (University of Bern); Schimanski, Bernd (University of Bern); Jakob, Martin (University of Bern); Schnarrwiler, Felix (University of Bern); Schneider, Andre (University of Bern); Schnaufer, Achim (University of Edinburgh); Zuber, Benoit (University of Bern); Ochsenreiter, Torsten (University of Bern)*

The mitochondrial genome and its baroque organization into a large network, the so-called kinetoplast, provided the name for our favourite model organisms. While more than 30 proteins involved in the replication of the kDNA have been characterized mainly in Crithidia and Trypanosoma brucei . Over the last two decades the molecular basis and dynamics of the mitochondrial genome segregation machinery the so called tripartite attachment complex (TAC) remained largely unknown. In the last seven years, three proteins and a monoclonal antibody have been described at different levels of detail. We have identified a novel component of the TAC (TAC102) that based on RNAi, super-resolution microscopy (STED), biochemical (monoclonal antibody) as well as ultrastructural analysis belongs to the core machinery of the TAC.
Furthermore we analysed the hierarchy of the individual components in the TAC and describe the dynamics of the complex during kDNA segregation. Based on this data we present a model of TAC replication in T. brucei .

2E A new model for regulation of flagellum length in Trypanosoma brucei

Bertiaux, Eloise (Institut Pasteur)*; Morga, Benjamin (Institut Pasteur); Perrot, Sylvie (Institut Pasteur); Rotureau, Brice (Institut Pasteur); Bastin, Philippe (Institut Pasteur)*

During its complex life cycle, Trypanosoma brucei undergoes significant morphological changes including extensive variations in flagellum length (3 to 30 µm). Here, we searched to understand the mechanism controlling flagellum length. Intraflagellar transport (IFT) refers to the movement of protein complexes between the membrane and the flagellar microtubules and is essential for the construction of the flagellum by bringing tubulin to the assembly site. It could therefore be a central element in the definition of flagellum length. We show that the total amount of IFT proteins is directly related to the length of the flagellum. This could be explained if the total amount of IFT was injected at once at the start of flagellum construction. However our data reveal that IFT proteins are regulaly injected in the flagellum during elongation whilst maintaining a constant concentration per unit length. To evaluate whether IFT availability could regulate flagellum length, the IFT kinesins were knockdowned leading to a decrease in frequency and speed of IFT, a phenomenon accompanied by construction of a flagellum that is too short (7 µm) . Nevertheless flagellum elongation continues after cell division (12 µm) , until the trypanosome restart the cell cycle. We propose that flagellum length is controlled by a combination of IFT availibility and timing of cell cycle. This model can explain the production of flagella of different length during the parasite cycle.

2F Proteomic identification of novel cytoskeletal proteins associated with TbPLK, an essential regulator of cell morphogenesis in T. brucei

de Graffenried, Christopher L. (Brown University)*; McAllaster, Michael (Brown University); Ikeda, Kyojiro (MFPL); Lozano-Nunez, Ana (MFPL); Anrather, Dorothea (MFPL); Unterwurzacher, Verena (MFPL); Gossenreiter, Thomas (MFPL); Perry, Jenna (Brown University); Vaughan, Sue (Oxford Brookes University)*

Trypanosoma brucei is the causative agent of African sleeping sickness, a devastating disease endemic to Sub-Saharan Africa with few effective treatment options. The parasite is highly polarized, including a single flagellum that is nucleated at the posterior of the cell and adhered along the cell surface. These features are essential and must be transmitted to the daughter cells during division. Recently, we have identified the T. brucei homolog of polo like kinase (TbPLK) as an essential morphogenic regulator. We have now conducted proteomic screens to identify potential TbPLK binding partners and substrates to better understand the molecular mechanisms of kinase function. These screens identified a cohort of proteins, most of which are completely uncharacterized, which localize to key cytoskeletal organelles involved in establishing cell morphology, including the flagella connector, flagellum attachment zone, and the bilobe structure. Depletion of these proteins causes substantial changes in cell division, including mispositioning of the kinetoplast, loss of flagellar connection, and prevention of cytokinesis. The proteins identified in these screens provide the foundation for establishing the molecular networks through which TbPLK directs cell morphogenesis in T. brucei.

2G Architecture of a host-parasite interface: complex targeting mechanisms revealed through proteomics

Gadelha, Catarina (University of Nottingham)*; Zhang, Wenzhu (The Rockefeller University); Wickstead, Bill (University of Nottingham); Chait, Brian (The Rockefeller University); Field, Mark (University of Dundee)*

Surface membrane organization and composition are key to parasite growth, immune evasion and transmission, with surface-exposed proteins that represent promising therapeutic targets. For extracellular African trypanosomes, the surface is partitioned such that all endo- and exocytosis is directed through a specific membrane region, the flagellar pocket, in which it is thought the majority of invariant surface proteins reside. However, very few of these proteins have been identified, severely limiting functional studies, and hampering the development of potential treatments. Here we used an integrated biochemical, proteomic and bioinformatic strategy to identify surface components of the human parasite Trypanosoma brucei. This ‘surfeome’ contains previously known flagellar pocket proteins as well as multiple novel components, and is significantly enriched in proteins that are essential for parasite survival. Molecules with receptor-like properties are almost exclusively parasite-specific, whereas transporter-like proteins are conserved in model organisms. Validation shows that the majority of surfeome constituents are bona fide surface-associated proteins, and as expected, the majority present at the flagellar pocket. Moreover, the largest systematic analysis of trypanosome surface molecules to date provides evidence that the cell surface is compartmentalized into three distinct domains with free diffusion of molecules in each, but selective, asymmetric traffic between. This work provides a paradigm for the compartmentalisation of a cell surface and a resource for its analysis.

2H Exosome secretion of spliced leader RNA is a novel sensing mechanism in trypanosomes

Michaeli, Shulamit (Bar-Ilan University); *Eliaz, Dror (Bar-Ilan University); Shaked, Hadassa (Bar-Ilan University); Arvatz, Gil (Bar-Ilan University); Tkacz, Itai Dov (Bar-Ilan University); Binder, Lior (Bar-Ilan University); Waldman Ben Asher, Hiba (Bar-Ilan University)*

The biogenesis of the spliced leader RNA (SL RNA) that is donated to all mRNAs by trans-splicing in trypanosomatids, is complex. The SL RNA undergoes co-transcriptional specific modifications of the cap and is assembled to an RNP complex, in SL RNP factory (1). When SL RNA biogenesis is blocked in cells silenced for Sm proteins, SMN, or GEMIN2 factors (2, 3) or under heat-shock SL RNA accumulates first in the nucleus and then migrates to the cytoplasm to form distinct cytoplasmic "speckles" designated SL RNP-C. In this study, we purified the SL RNP-C complex and identified its binding proteins by mass-spectrometry. The complex is not similar to any known complex described so far. One of the proteins, a p58 RNA helicase was chosen as a hallmark of the complex. Multivesicular bodies carrying exosomes containing p58 were detected by cryo-EM microscopy. p58 and SL RNA are secreted by exosomes using the endosomal sorting machinery ESCRT and silencing of Vps36 which controls the secretion of microRNA in mammals, abolished the secretion of SL RNA. But why would trypanosomes stabilize SL RNA, only to subsequently dispose of it. Secretion of SL RNA via exosomes affect the social motility of procyclic parasites. We suggest that SL RNA secretion is a signal that can potentially control parasite migration in the insect host. This is the first study to suggest a communication function for T. brucei exosomes similar to their role in balancing malaria infection (4). 1. Michaeli S (2011) Future Microbiology 4, 459-74 2. Mandelboim et al., (2003) J. Biol. Chem. 278, 51469-51478 3. Tkacz et al., (2010) J. Biol. Chem 285, 27982-27999 4. Regev-Rudzki, N. et al., (2013) Cell 153, 1120-1133

SESSION III: Nucleus/Kinetoplast/Gene Expression

Laurie Read, Chair.

Sunday 2:00pm

04/26/2015

3A Conserved functions in the replication and segregation of nuclear and kinetoplast DNA provide a potential communication route between the two genomes in T. brucei

Shlomai, Joseph (Department of Microbiology and Molecular Genetics The Hebrew University of Jerusalem)*; Klebanov-Akopyan, Olga (Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem); Yaffe, Nurit (Department of Microbiology and Molecular Gennetics, The Hebrew University of Jerusalem); Glousker, Galina (Department of Genetics, The Hebrew University of Jerusalem); Kapach, Guy (Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem); Dvir, Rotem (Institute of Chemistry, The Hebrew University of Jerusalem); Porath, Danny (Institute of Chemistry, The Hebrew University of Jerusalem); Tzfati, Yehuda (Department of Genetics, The Hebrew University of Jerusalem)*

The universal minicircle sequence (UMS), conserved at the origins of kinetoplast DNA (kDNA) minicircles is bound by the protein UMSBP. This protein decondenses in vitro kDNA networks, condensed by the kinetoplast associated proteins (KAPs), through its protein-protein interactions with KAPs. kDNA condensation was studied in details and 3D, using atomic force microscopy, revealing its proceeding through generation of stacks of DNA circles and their hierarchical assembly into higher order condensation centers. The two distinct UMSBP proteins of T. brucei, a kinetoplast-associated TbUMSBP1 and a nuclear TbUMSBP2, display both the origin binding and kDNA decondensation activities. Knockdown analyses indicated that TbUMSBP2 plays a role in nuclear DNA metabolism, whereas both proteins are required for kDNA replication and segregation. The two proteins interact with each other, forming heterodimers, which may be in accord with their mutual mitochondrial functions. Study of TbUMSBP2's nuclear function revealed that the protein binds in vitro not only to the mitochondrial UMS but also to the G-rich single-stranded chromosomal telomeric sequence, and colocalizes in vivo with telomeres at the nuclear periphery. Knockdown of TbUMSBP2 resulted in impaired nuclear division, endoreduplication and cell growth arrest. Strikingly, depletion of TbUMSBP2 caused delocalization of telomeres and induced the phosphorylation of histone H2A foci, indicating that the telomere capacity to suppress DNA damage repair (DDR) was compromised. The similarity in G-rich UMS and telomeric sequences and their specific binding proteins between the nucleus and the mitochondrion may indicate a common evolutionary origin and a conserved function in DNA replication and segregation between the two genomes and may also point on a potential communication route between the two organelles in T. brucei.

3B Direct monitoring of stepwise folding of the kinetoplast DNA network

Yaffe, Nurit (The Hebrew University of Jerusalem)*; Rotem, Dvir (The Hebrew University of Jerusalem); Porath, Danny (The Hebrew University of Jerusalem); Shlomai, Joseph (The Hebrew University of Jerusalem)*

Packaging and condensation of genomes into prokaryotic and mitochondrial nucleoids and nuclear chromatin, affect genome structure, replication and expression. In the case of trypanosomatids the remarkable kinetoplast DNA (kDNA) network resides in the mitochondrial matrix in a disc-shape nucleoid condensed by histone H1-like proteins (KAPs). We found specific protein-protein interactions between the kinetoplast DNA replication initiator protein UMSBP and two of the histone H1-like proteins KAP3 and KAP4. Using the high resolution of atomic force microscopy (AFM), which allows analyses at the nano scale, with neither fixation nor metal coating, we were able to visualize kDNA networks in details through the condensation process. Analyses of kDNA condensation intermediates revealed that kDNA condensation proceeds via sequential hierarchical steps. Networks condensation intermediates display similar perimeter to those of uncondensed networks, but with remarkable differences in their internal structure and organization. Multiple local condensation foci (LCF), each containing dozens of DNA circles, are generated, with minor changes in the networks' perimeter, forming a network of condensation foci, which are interconnected by DNA fibers. Upon further condensation, multiple LCF assemble into few higher order condensation centers, leading to full network condensation. The kDNA folding pathway described here may shed light on a basic folding mechanism that mediate condensation and packaging of kDNA and may be applied to DNA folding in other cells and organelles.

3C Coupled 3'-5' degradation and 3' uridylation constitute the major RNA processing pathway in mitochondria of trypanosomes

Afasizhev, Ruslan (Boston University)*; Suematsu, Takuma (Boston University); Zhang, Liye (Boston University); Monti, Stefano (Boston University); Afasizheva, Inna (Boston University)*

In Trypanosoma brucei, mitochondrial RNAs are produced by sequential nucleolytic degradation of multicistronic precursors, 3' adenylation, U-insertion/deletion editing and post-editing 3' adenylation/uridylation. RNA editing reactions are directed by guide RNAs which are also generated from long precursors and 3' uridylated. We previously demonstrated that repression of the terminal uridyltransferase (TUTase) RET1 leads to a loss of 3' oligo(U) tails and accumulation of gRNA and mRNA precursors. The former observation is consistent with RET1's activity while the latter finding remained puzzling because the enzyme lacks nuclease activity. In this work, we show that RET1 forms a stable stoichiometric complex with the 3'-5' exonuclease DSS1 and several proteins lacking any discernible motifs. We designated this complex as 3' processome. Repression of DSS1 led to a loss of mature gRNAs and accumulation of gRNA precursors indicating that RET1 and DSS1 function in the same processing pathway. Purified DSS1 is catalytically inactive, but as a subunit of the 3' it degrades single-stranded RNA and structured RNAs to 4-5 nt oligonucleotides. However, in vivo degradation stops precisely at the 3' ends of gRNAs, mRNAs and rRNAs. Deep sequencing of small mitochondrial RNAs indicated that guide RNA-sized antisense transcripts may define the 3' boundaries of mature mitochondrial RNAs by blocking 3'-5' precursor degradation. This processes was reconstituted in vitro to demonstrate that RNA uridylation simulates 3' processome recruitment to RNA precursors and that extended double-stranded regions are indeed required to block the degradation. Collectively, our data indicate that coupling of 3'-5' degradation and 3' uridylation is achieved by confining RET1 and DSS1 to the same particle. Furthermore, uridylation-triggered degradation represents the main RNA processing pathway in mitochondria of trypanosomes.

3D Maintaining the mitochondrial membrane potential in bloodstream and dyskinetoplastic T. brucei: a game of two players.

Zikova, Alena (Biology Centre)*; Veselikova, Michaela (Faculty of Science, University of South Bohemia); Subrtova, Karolina (Faculty of Science, University of South Bohemia); Panicucci, Brian (Biology Centre)*

The infective bloodstream stage (BS) of Trypanosoma brucei possesses a single mitochondrion that lacks a cytochrome-mediated respiratory chain and thus employs the hydrolytic activity of FoF1-ATPase to maintain the essential mitochondrial (mt) membrane potential (??m). Meanwhile, dyskinetoplastic (Dk) trypanosomes lacking the mt encoded A6 that is essential for the functional Fo proton pore alternatively maintain their ??m by combining the hydrolytic activity of the matrix-facing F1-ATPase and the electrogenic exchange of ATP4- for ADP3- by the ADP/ATP carrier (AAC). Notably, we have shown that in addition to the expected presence of F1-ATPase, membrane-bound FoF1 complexes are also present and important for Dk cell growth [1]. To explore how BS T. brucei would cope with a reduction in functional A6, we generated a mt peptide release factor knock-out cell line (?TbMrf1) that is viable, but has a slightly increased doubling time compared to wild type BS parasites and is unable to establish a lethal infection in BALB/c mice. Interestingly, interfering with the mt translation machinery results in cells with a decreased ??m, a decreased abundance of FoF1-ATPase and an increased sensitivity to both FoF1-ATPase and AAC inhibitors. This suggests that in ?TbMrf1 cells, a sufficient ??m for in vitro growth is provided through the coordinated efforts of the efficient proton pumping of the remaining FoF1-ATPase and the electrogenic exchange created by AAC. This is in contrast to the relatively low sensitivity of wild type BS trypanosomes to AAC inhibitors and indicates that there might be an alternative source of mt ATP, which calls for revisiting the mt energy metabolism in the infectious stage of this parasite. [1] Šubrtová, Panicucci, Zíková. Plos Pathogens, in press

3E Transposable elements in the Crithidia fasciculata genome: a potential role in chromosome rearrangement and transcription

Myler, Peter J. (Seattle BioMed)*; Ramasamy, Gowthaman (Seattle BioMed); Steinbiss, Sascha (Wellcome Trust Sanger Institute); Hertz-Fowler, Christiane (University of Liverpool); Tomlinson, Chad (The Genome Institute at Washington University); Warren, Wes (The Genome Institute at Washington University); Akopyants, Natalia (Washington University School of Medicine); Beverley, Stephen (Washington University School of Medicine)*

As part of the NHGRI/NIAID Kinetoplastid White Paper agreement to sequence and annotate 22 Leishmania and Trypanosoma genomes, we used SMRT (PacBio) sequencing to generate a nearly complete assembly of the Crithidia fasciculata genome. There appear to be only 31 chromosome pairs, with considerable allelic heterozygosity. About a third of the Crithidia chromosomes are essentially syntenic with their counterparts in Leishmania, while the remainder show one to three breaks in synteny compared to the corresponding Leishmania chromosomes. The gene content of Crithidia appears quite similar to that of Leishmania, although there are numerous cases of Crithidia-specific gene expansion and some instances of genes apparently acquired by horizontal transfer from bacteria. All Crithidia chromosomes contain two different transposable elements (TATEs and TAREs) at one or both telomeres, and many also contain a sub-telomeric gene encoding a leucine rich repeat protein (TALRRP). Most of the remaining chromosome ends contain tandem arrays of 19- to 21-nt repeats, which also bound the ribosomal RNA locus. Many chromosomes also contain internal retrotransposons related to the Ingi/L1Tc elements found in Trypanosoma. Interestingly, these are usually found in regions associated with transcription initiation and termination in Leishmania and Trypanosoma, often corresponding to synteny breaks between genomes. These results suggest a role for transposable elements in driving trypanosomatid chromosome rearrangement and the unusual organization of protein-coding genes into polycistronic transcription units. SMRT (PacBio) sequencing also allowed us to map the location of the trypanosomatid-specific base J, which we speculate may have arisen as a mechanism for silencing transcription of retrotransposons.

3F Bromodomain proteins affect multiple immune evasion mechanisms and maintain life-cycle stage identity in trypanosomes

Schulz, Danae (Rockefeller University)*; Debler, Erik (Rockefeller University); Mugnier, Monica (Rockefeller University); Kim, Hee-Sook (Rockefeller University); Papavasiliou, Nina (Rockefeller University)*

Trypanosoma brucei is transmitted to its mammalian host by the tsetse, Glossina morsitans. In the fly, the parasite's surface is covered with procyclin, encoded by the EP and GPEET genes, while in the mammal it is densely covered with Variant Surface Glycoprotein (VSG). The parasite relies on two strategies to evade the mammalian host antibody response. First, the parasite varies its highly immunogenic surface VSG, using a repertoire of ~2500 distinct VSG genes. Second, host antibody bound to VSG is rapidly internalized by the parasite. Using both small molecule inhibitors and genetic mutants for individual proteins, we find that trypanosome bromodomain proteins that recognize acetylated lysine residues are important for both aspects of immune evasion, as their inhibition perturbs both monoallelic expression of VSG genes and internalization of surface-bound antibodies. We have also found that bromodomain proteins are important for maintaining cell identity as bloodstream form (BF) cells. First, bromodomain inhibition results in expression of EP1 on the cell surface. Second, RNA-seq reveals changes in the transcriptome similar to those seen in differentiating cells. This study reveals an unexpected role for trypanosome bromodomain proteins in both immune evasion and the maintenance of life-cycle stage identity. Importantly, bromodomain inhibition leads to a decrease in virulence in vivo, suggesting that these proteins could be therapeutic drug targets for trypanosomiasis. Our 1.25Å resolution crystal structure of a trypanosome bromodomain in complex with a known acetyl-lysine mimetic reveals a novel binding mode of the inhibitor, which serves as a promising starting point for rational drug design.

SESSION IV:Biochemistry/Drugs

Michael Boshart, Chair.

Monday 8:45am

04/27/2015

4A Arginine sensing pathway in Leishmania induces a Mitogen Activated Protein Kinase 2-mediated response during macrophage invasion

Zilberstein, Dan (Technion-Israel Institute of Technology)*; Goldman, Adele (Technion-Israel Institute of Technology); Balno, Caitlin (Technion-Israel Institute of Technology); Strasser, Rona (McGill University); Rentsch, Doris (Bern University); Myler, Peter, J. (Seattle BioMed); Jardim, Armando (McGill University); Wiese, Martin (Glasgow University)*

Protozoan of the genus Leishmania are the causative agents of leishmaniasis in humans. These parasites cycle between promastigotes in the sand fl y mid-gut and amastigotes in phagolysosome of mammalian macrophages. During infection, parasites up-regulate macrophage nitric oxide synthase and arginase activity, both of which use arginine as a substrate. These elevated activities depleted macrophage arginine pools, a situation that invading Leishmania cannot tolerate as this is an essential amino acid. Leishmania donovani imports exogenous arginine via a mono-specific amino acid transporter (AAP3) and utilizes it primarily through the polyamine pathway to provide precursors for trypanothione biosynthesis. Here we report the discovery of an arginine availability pathway that senses the lack of environmental arginine . Depletion of arginine from promastigote and amastigote growth media induces a rapid up-regulation in AAP3 expression and activity, as well as a few other genes. Significantly, this arginine availability pathway is also activated in parasites during macrophage infection . Phosphoproteomic analyses of L. donovani promastigotes have implicated a mitogen activated protein kinase 2 (MPK2) -mediated signaling cascade in this response and L. mexicana mutants lacking MPK2 are unable to respond to arginine deprivation. In addition, these mutants cannot differentiate axenically into amastigotes and do not survive as amastigotes in peritoneal macrophages, and failed to establish an infection in mice. We propose that sensing arginine levels plays a critical role in Leishmania virulence by activating a rapid metabolic reaction for salvaging this amino acid in response to the lower arginine concentration in the macrophage phagolysosome. This response further promotes amastigote survival by further depleting the macrophage arginine pool, thereby suppressing production of cytotoxic nitric oxide.

4B Structural characterisation of a novel family of GPI-anchored surface glycoproteins expressed in the metacyclic stage of Trypanosoma brucei

Acosta-Serrano, Alvaro (Liverpool School of Tropical Medicine, England.);
Casas-Sánchez, Aitor (Liverpool School of Tropical Medicine); Fisher, Lewis (Liverpool School of Tropical Medicine); Jackson, Andrew (University of Liverpool); Boulanger, Martin (University of Victoria)

Development of Trypanosoma brucei within the tsetse vector is accompanied by the expression of stage-specific families of glycosylphosphatidyl inositol (GPI) -anchored surface glycoproteins. While (midgut) procyclics sequentially express GPEET- and EP-procyclins, Brucei Alanine-Rich Proteins (BARP) and variant surface glycoproteins (VSG) are so far the only surface markers described for the epimastigote and metacyclic stages, respectively. In a recent proteomic analysis of saliva from T. brucei -infected tsetse flies (Perally S. et al., in preparation), we found that it is particularly enriched with several trypanosome surface molecules, including BARP, VSG and a novel family of hypothetical GPI-anchored surface glycoproteins previously designated as “Clade IV”. Clade IV belongs to the large family (Fam50) of trypanosome surface glycoproteins that include BARP and, T. congolense GARP and CESP. T. brucei Clade IV proteins are encoded by a small (five) family of genes, which are exclusively expressed in the metacyclic stage and have products with 90% identity, varying only in the number of amino acid repeats (up to three) at their C-termini. In order to gain insights into the function of Clade IV glycoproteins, we expressed one of the paralogs (Tb927.7.360) in Sf9 cells and determined its crystal structure at 1.9 Å resolution. Tb927.7.360 adopts an extended helical bundle structure with an overall shape that highly resembles that of VSG and GARP despite their high degree of sequence divergence. We postulate that one function of Clade IV proteins may be to maintain the tight intramolecular packing with VSG molecules on the metacyclic surface, which may be essential to transition into the vertebrate host. We are currently determining the essentiality and exact localisation of Clade IV proteins on the trypanosome surface.?

4C Metabolic signals and glycosomal isocitrate dehydrogenase (IDHg) control procyclic to metacyclic development of Trypanosoma brucei fly stages

Boshart, Michael (Faculty of Biology, Genetics, Ludwig-Maximilians-University Munich, Martinsried, Germany)*; Allmann, Stefan (Faculty of Biology, Genetics, Ludwig-Maximilians-University Munich, Martinsried, Germany); Ziebart, Nicole (Faculty of Biology, Genetics, Ludwig-Maximilians-University Munich, Martinsried, Germany); Dupuy, Jean-William (Centre de Génomique Fonctionnelle, Plateforme Protéome, Université de Bordeaux, Bordeaux, France); Bonneu, Marc (Centre de Génomique Fonctionnelle, Plateforme Protéome, Université de Bordeaux, Bordeaux, France); Bringaud, Frédéric (Centre de Résonance Magnétique des Systèmes Biologiques (RMSB), UMR5536, Université de Bordeaux, CNRS, Bordeaux, France); Van De Abbeele, Jan (Institute of Tropical Medicine Antwerp (ITM), Dept. Biomedical Sciences, Antwerp, Belgium)*

Metabolic adaptation of trypanosomes to changing host environments and carbon source availability is established for bloodstream and fly gut conditions. In contrast, the life cycle segment within the tsetse is a black box in this respect. Using the RBP6-driven culture development (Kolev et al., 2012), we have investigated the impact of carbon source availability on procyclic to metacyclic development. Glucose withdrawal significantly accelerated, and glycerol inhibited development in this system, suggesting that these carbon sources act as metabolic signals for development in the fly. Global proteome analysis has specifically identified a set of enzymes of citrate metabolism (citrate synthase, aconitase, glycosomal isocitrate dehydrogenase, IDHg) being induced by minus glucose conditions and repressed by glycerol. Metabolite analysis of a series of knock out lines deficient in these enzymes provides evidence for a novel pathway feeding glycosomal NADPH production by IDHg. This seems to compensate the reduced activity of the pentose phosphate pathway in minus glucose conditions. When the ∆idhg/∆idhg mutant was tested in the RBP6-driven system, development arrested in the epimastigote stage and no metacyclic forms were detected. Control mutants were unaffected. In agreement, fly passage of the ∆idhg/∆idhg mutant revealed a dramatic and rescueable maturation phenotype with normal midgut infections, but sterile salivary glands. IDHg plays an essential role for development in the tsetse, hence the upregulation (by glucose withdrawal) of a novel pathway to feed this enzyme. Furthermore, mutant analysis in the RBP6-driven system has been validated by fly passage for the first time to our knowledge. Currently, the developmental role of possible pathways utilizing the IDHg-generated reduced coenzymes in glycosomes is investigated.

4D Cell surface proteomics yields insight into stage-specific remodeling of the host-parasite interface in Trypanosoma brucei

Shimogawa, Michelle (UCLA)*; Saada, Edwin (UCLA); Vashisht, Ajay (UCLA); Wohlschlegel, James (UCLA); Hill, Kent (UCLA)*

African trypanosomes are devastating human and animal pathogens transmitted by tsetse flies between mammalian hosts. The trypanosome surface forms a critical host interface that is essential for sensing and adapting to diverse host environments and additionally impacts success of therapeutics. However, trypanosome surface protein composition and diversity remain largely unknown. Here, we use surface labeling, affinity purification and proteomic analyses to describe cell surface proteomes from insect and mammalian bloodstream-stage Trypanosoma brucei. Analysis of multiple independent replicates and label-free quantitation of intracellular and surface protein controls indicate that the surface proteomes reported here represent high-confidence datasets of candidate surface proteins. We identify most known surface proteins, as well as many novel proteins of unknown function. Notably, we demonstrate stage-specific expression for paralogues within several large protein families. This indicates that fine-tuned remodeling of the parasite surface allows adaptation to diverse host environments, while still fulfilling universally essential cellular needs. Our surface proteome analyses complement transcriptomic, proteomic and in silico analyses by highlighting proteins that comprise the host-parasite interface and are expected to be accessible to small molecule-based therapeutics.

4E Heme starvation controls proliferation and activate differentiation by inhibiting translation initiation regulation in Trypanosoma cruzi

Moretti, Nilmar (UNIFESP)*; Augusto, Leonardo (UNIFESP); Schenkman, Sergio (Universidade Federal de Sao Paulo)*

Heme is not synthesized by trypanosomes. It has to be scavenged from the host for growth. The availability of heme is variable in Trypanosoma cruzi and we hypothesised that it could be a key factor in controlling the developmental changes during this parasite life cycle. Here we characterized a protein-kinase that it is activated in the absence of heme. This kinase, named TcK2 phosphorylates the alpha subunit of the translation initiation factor 2 (eIF2α), a key protein involved in eukaryote translation control by inducing stress remedial responses. TcK2 was found located in membranes of organelles that accumulate nutrients in proliferating parasite forms. The heme binds specifically to the catalytic domain of this kinase, inhibiting its activity. In the absence of heme, TcK2 is activated, arresting cell growth and inducing differentiation of proliferative into infective and non-proliferative forms. Parasites lacking TcK2 lose this differentiation capacity and heme is not stored in reserve organelles, remaining in the cytosol. This results in the accumulation of hydrogen peroxide, because of increased superoxide dismutase and decreased peroxidase levels, which drives the generation of reactive oxygen species. These phenotypes could be reverted by the re-expression of the wild type but not of a TcK2 dead mutant. We concluded that heme is a key factor controlling the protein synthesis during parasite proliferation. Differentiation induction might occur through a preferential protein synthesis combined with unbalanced levels of reactive oxygen species.
FAPESP/CNPq

4F cAMP response proteins (CARPs) regulates downstream signalling of cAMP in Trypanosoma brucei

Tagoe, Daniel N. (Wellcome Trust Centre for Molecular Parasitology and Institute of Infection, Immunity and Inflammation, University of Glasgow)*; Gould, Matt (Biocenter, Genetics, Ludwig-Maximillians-University of Munich, Martinsried, Germany); Kalejaiye, Titilola (Institute of Infection, Immunity and Inflammation, University of Glasgow); Bachmaier, Sabine (Biocenter, Genetics, Ludwig-Maximillians-University of Munich, Martinsried, Germany); Boshart, Michael (Biocenter, Genetics, Ludwig-Maximillians-University of Munich, Martinsried, Germany); de Koning, Harry P. (Institute of Infection, Immunity and Inflammation, University of Glasgow)*

Current drug treatment options in trypanosomiasis are old, toxic and highly ineffective. Signal transduction pathways have been shown to have essential cellular functions and have therefore often been exploited as pharmacological targets. The metabolism of cyclic adenosine monophosphate (cAMP) has recently been validated as a drug target in T. brucei. The phosphodiesterase inhibitor CpdA, used in the validation, was fatal to bloodstream forms. However, the downstream effector proteins of cAMP activity in trypanosomes are unknown. We use reverse genetics, genomics and proteomics to elucidate potential pathways sensitive to changes in cellular cAMP levels. An RNAi library screen identified novel cA MP R esponse P roteins (CARPs), which are mainly unique to kinetoplastids. A single allele deletion of any of the CARPs increases resistance to CpdA whilst attempted double deletions have not been possible so far – indicating that most of these genes are essential. Extensive characterization shows all the CARPs are involved in the cAMP response individually or in a complex. Particularly, CARP3 seems to be an important determinant of cAMP activity, as its over-expression limits growth, and significantly increased both internal cAMP levels and efflux of cAMP from the cells. Deep sequencing (RIT-Seq) of T. brucei expressing an RNAi library (RIT-Seq), after exposure to CpdA, has delineated several new potential CARPs, including protein phosphatases and adenylyl cyclases. CARP3 is trypanosome-specific thus providing a potentially unique therapeutic target for drug development as well as elucidating the biochemistry of cAMP signalling in trypanosomes.

4G Kinetoplastid-specific pATOM36 mediates membrane insertion of a subset of mitochondrial outer membrane proteins

Käser, Sandro (University of Bern)*; Oeljeklaus, Silke (University of Freiburg); Warscheid, Bettina (University of Bern); Schneider, André (University of Bern)*

In trypanosomes, as in other eukaryotes, more than 95% of all mitochondrial proteins are imported into the mitochondrion. The recently characterized multisubunit ATOM complex mediates import of essentially all proteins across the outer mitochondrial membrane in T. brucei . Moreover, an additional protein termed pATOM36, which is loosely associated with the ATOM complex, has been implicated in the import of only a subset of mitochondrial matrix proteins. Here we have investigated more precisely which role pATOM36 plays in mitochondrial protein import. RNAi mediated ablation of pATOM36 specifically depletes a subset of ATOM complex subunits and as a consequence results in the collapse of the ATOM complex as shown by Blue native PAGE. In addition, a SILAC-based global proteomic analysis of uninduced and induced pATOM36 RNAi cells together with in vitro import experiments suggest that pATOM36 might be a novel protein insertase acting on a subset of alpha-helically anchored mitochondrial outer membrane proteins. Identification of pATOM36 interaction partners by co-immunoprecipitation together with immunofluorescence analysis furthermore shows that unexpectedly a fraction of the protein is associated with the tripartite attachment complex (TAC). This complex is essential for proper inheritance of the kDNA as it forms a physical connection between the kDNA and the basal body of the flagellum throughout the cell cycle. Thus, the presence of pATOM36 in the TAC provides an exciting link between mitochondrial protein import and kDNA inheritance.

4H Chemical biology uncovers mechanisms for controlling invariant surface glycoprotein expression in Trypanosoma brucei

Zoltner, Martin (Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee)*; Leung, Ka Fai (Department of Pathology, University of Cambridge, Cambridge); Alsford, Sam (London School of Hygiene and Tropical Medicine, Keppel Street, London); Horn, David (Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee); Mark, Field (Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee)*

The anti-trypanosomal drug suramin was developed a century ago and remains in use as early stage treatment. The mode of action of suramin remains elusive. Using RITseq we identified a large cohort of gene products involved in suramin sensitivity. Significantly, the invariant surface glycoprotein ISG75 and two ubiquitin hydrolases Ubh1 and Ubh2 were identified, which are orthologs of ScUbp15/HsHAUSP1 and pVHL-interacting deubiquitinating enzyme 1 (type I) respectively. Recently we demonstrated that ISG internalisation and turnover is ubiquitylation dependent. Ubh1 knockdown reduced ISG75 but not ISG65 abundance, whilst both are decreased by Ubh2 knockdown. Furthermore, Ubh1, but not Ubh2, knockdown led to an enlargement of the flagellar pocket, consistent with a blockade in endocytosis. To understand the level of specificity in this system, we analysed the impact of Ubh1 and Ubh2 on the trypanosome proteome. Interestingly, for Ubh2 knockdown the only significantly affected proteins are ISG65 and ISG75, indicating a very precise and coupled control of ISG expression level. By contrast, Ubh1 knockdown perturbs a cohort of integral membrane proteins, including the acid phosphatase MBAP1 and additional ISG-related proteins. Further we find an increase in transferrin receptor and ESAG5, likely a result of inhibition of endocytosis by MBAP1. These data demonstrate a sophisticated and highly specific role for ubiquitin hydrolases in the control of trypanosome surface protein expression.

SESSION V: Pathogenesis

Barbara Burleigh, Chair.

Tuesday 8:45am

04/28/2015

5A Host and parasite genetics shape a link between tissue-specific Trypanosoma cruzi infection dynamics and cardiac pathogenesis

***Lewis, Michael D. (LSHTM)**; Fortes Francisco, Amanda (LSHTM); Taylor, Martin (LSHTM); Jayawardhana, Shiromani (LSHTM); Kelly, John (LSHTM)*

A major limitation of experimental models of Chagas disease has been the lack of sensitive tools for detecting tissue-specific parasite foci during chronic stage infections. Consequently, the influence of host-parasite interaction on the development and severity of cardiomyopathy has proven difficult to address. We developed highly sensitive bioluminescence imaging models using Trypanosoma cruzi expressing a firefly luciferase engineered to emit tissue-penetrating orange-red light. The system enabled long-term serial evaluation of parasite burdens in individual mice, with an in vivo detection limit of 100-1000 parasites. Ex vivo imaging allowed tissue-specific quantification of parasite loads with minimal sampling bias. To investigate chronic infection dynamics in the context of host and parasite genetic diversity, we compared four T. cruzi strains representing the TcI and TcVI subtypes in BALB/c, C3H/HeN and C57BL/6 mice, which carry MHC haplotypes H2d, H2k and H2b, respectively. In all host-parasite combinations, the gastrointestinal tract was the only persistently infected site. The frequency of infection foci in non-gut sites varied between models, being rare in TcVI-infected C57BL/6 and common in TcI-infected C3H/HeN. There was no evidence for preferential parasitism of the heart over other sites not associated with disease, such as the lung. Quantitative histopathological analysis revealed significant cardiac fibrosis in all host-parasite combinations. Disease severity correlated with the model-dependent frequency of non-gut infection foci, but not heart-specific end-point parasite loads or myocarditis intensity. These data identify the gut as the primary reservoir site and suggest that cardiomyopathy stems from sporadic trafficking of parasites into the heart, which occurs at a frequency determined by a combination of host and parasite genetics.

5B Metacyclogenesis of Trypanosoma cruzi Includes Starvation-Induced Transient Accumulation of Mitochondrial Gene Transcripts

Shaw, Aubie K. (University of Minnesota)*; Zimmer, Sara (University of Minnesota)*

Trypanosoma cruzi epimastigotes replicate and progress through the insect digestive tract. As nutrients are depleted, epimastigotes differentiate to infectious, non-replicative metacyclic trypomastigotes. Trypanosome developmental remodeling includes metabolic changes in the mitochondria. We sought to address whether life stage-specific regulation of mitochondrial-encoded gene products is part of T. cruzi developmental remodeling. Using insect stage axenic culture differentiation models, we analyzed mitochondrial gene expression in epimastigotes during metacyclogenesis. We found that mitochondrial RNAs increase as insect stage T. cruzi undergoes changes leading to infectivity. Specifically, abundances of all eight measured mitochondrial transcripts increased once epimastigotes depleted medium nutrients, but prior to completion of metacyclogenesis. Increases were largely abrogated in cultures containing a substantial portion of fully differentiated metacyclic trypomastigotes. Partial partitioning of the two cell stages from this culture allowed us to demonstrate that mitochondrial RNA abundance increases largely occur in epimastigotes "pre-adapted" for metacyclogenesis rather than in fully differentiated metacyclics. Glucose depletion of culture medium significantly precedes increases in mitochondrial transcript abundances; therefore, glucose levels alone do not drive this increase. However, loss of both glucose and amino acids results in rapid increases in transcript abundances. It is likely that multiple mechanisms regulate transcript abundance/stability, as transcripts exhibit different temporal abundance patterns, and we are currently investigating whether uridine insertion/deletion editing is responsive to starvation stimuli. Finally, increases in respiratory complex subunit mRNAs are specific to mitochondrially-encoded mRNAs. Collectively, our data demonstrate that epimastigotes respond to starvation by increasing mitochondrial transcript abundances. Transcript increases could drive increases in respiratory pathway complexes, which is potentially advantageous for continuous generation of metacyclics as the parasite population experiences starvation in the insect digestive tract.

5C L. donovani uses a bet hedging strategy that drives evolutionary adaptation through genome instability and dosage compensation

Späth, Gerald F. (Institut Pasteur)*; Prieto Barja, Pablo (Centre for Genomic Regulation (CRG)); Pescher, Pascale (Institut Pasteur); Kedra, Darek (Centre for Genomic Regulation (CRG)); Chaumeau, Victor (University Montpellier); Guerfali, Fatma (Institut Pasteur Tunis); Bastien, Patrick (University Montpellier); Sterkers, Yvon (University Montpellier); Notredame, Cedric (Centre for Genomic Regulation (CRG))*

Genotype-genotype and environment-genotype interactions during the Leishmania life cycle drive parasite evolution towards higher fitness with important consequences for pathogenesis, tropism, and drug susceptibility. We applied HTseq and DNA-FISH analyses on L. donovani LD1S hamster-isolated amastigotes to study genomics and transcriptomics consequences of culture adaptation as a proxy for parasite evolution in response to environmental change. In contrast to the LDBPK reference genome, LD1S amastigotes are disomic with the exception of chromosome 31, for which we confirmed a tetrasomic state in situ. Analysis of derived promastigotes at in vitro passages p2, p10 and p20 revealed highly dynamic genomic changes, including generation of extra-chromosomal amplicons and aneuploidies for chromosomes 5, 9, 14, 15, 20, 23, and 26 as early as at p2 (or 20 generations). Adaptation to high density in vitro growth was associated with important fitness costs causing permanent loss of parasite virulence likely as the result of irreversible genomic alterations incompatible with infectivity. DNAseq and RNAseq analyses of 8 sub-clones derived from p20 parasites uncovered a surprising diversity in the genomic and transcriptomic profiles and dissociated the ploidy state from transcript abundance for most amplified chromosomes, except chromosome 26. These results document a yet undefined mechanism of dosage compensation that operates in a chromosome-specific manner, and demonstrate the parasite capacity to attain equal fitness while maintaining genomic diversity. Quantification of the chromosome ploidy state in spleen- and liver-derived amastigotes ex vivo at the single cell level by DNA-FISH analysis confirmed the presence of mosaic aneuploidies in situ uncovering a bet hedging strategy adopted by Leishmania, where the generation of a wide genetic diversity drives parasite evolution towards higher fitness.

5D Genome-wide identification of negative regulators of stumpy-specific developmental gene expression

Rico Vidal, Eva (University of Edinburgh)*; Glover, Lucy (University of Dundee); Ivens, Al (University of Edinburgh); Horn, David (University of Dundee); Matthews, Keith (University of Edinburgh)*

During their differentiation from proliferative slender parasites to non-proliferative, transmissible stumpy forms in blood, African trypanosomes down-regulate the expression of many genes as they become quiescent in preparation for transmission. However, a small subset of genes is up-regulated, particularly the ESAG9 family whose mRNAs are highly elevated in intermediate and stumpy forms. ESAG9 proteins are secreted into the bloodstream, so although their function remains unknown, they could be potentially involved in host-parasite interactions and/or transmission. They also provide a model gene family for the understanding of slender and stumpy gene expression control. To understand how stumpy-enriched genes are held silent in slender forms, we have carried out a genome-wide reverse genetic screen in which a T. brucei whole genome RNAi library has been transfected into parasites expressing a neomycin resistance gene controlled by the ESAG9 3'UTR. Under elevated Geneticin selection, the screen selected parasites where ESAG9 3'UTR-mediated gene silencing was reduced, generating increased neomycin expression and therefore increased Geneticin resistance. The screen has successfully identified four negative regulators, all of them containing RNA binding domains, that operate through the ESAG9 3'UTR: DRBD5, ZFP3 and two newly characterised genes, REG9-1 and REG9-2. Individual RNAi of each of these genes reproduces the effect in neomycin observed with the library, whilst targeting the REG9-1 gene by RNAi in trypanosomes capable of the slender to stumpy transition results in de-repression of ESAG9 mRNA in slender forms. Our studies have identified the first RNA binding proteins regulating the expression of stumpy-enriched genes in the mammalian bloodstream.

5E Adipose tissue is where most Trypanosoma brucei parasites accumulate and a probable source of relapse after treatment

Trindade, Sandra (Instituto de medicina Molecular)*; Figueiredo, Luisa M. (Instituto de Medicina Molecular); Rijo-Ferreira, Filipa (Instituto de Medicina Molecular); Carvalho, Tania (Instituto de Medicina Molecular); Van Den Abbeele, Jan (Institute of Tropical Medicine Antwerp); Ribeiro, Ruy M (Los Alamos National Laboratory); Dias, Sergio (Instituto de Medicina Molecular)*

Relapses in sleeping sickness treated patients are believed to result from parasites present in the brain, which cannot be effectively eliminated by most drugs. It remains unknown if the brain is the exclusive source of relapse. Using a mouse model, we studied the distribution of Trypanosoma brucei in several organs throughout the course of infection by histology, electron microscopy and quantitative PCR. We observed that early after infection, foci of parasites are preferentially detected in the adipose tissue, in the interstitial spaces between adipocytes. This bias occurs when infection is initiated by the natural way through tsetse fly bite, as well as by artificial injection routes. It becomes more pronounced later in infection, in which there is over 10-times more parasites in adipose tissue than in the blood. Mutant obese mice (ob/ob and db/db) showed an even higher parasite load in adipose tissue, indicating that the total parasite load in the host is dependent on its adipose mass. In mice treated with suramin, a drug commonly used to treat sleeping sickness, parasites persisted in the adipose tissue but not in other peripheral organs. Transplantation of gonadal adipose tissue from infected mice treated with suramin to recipient naïve mice resulted in infection of the latter, showing that there were still viable parasites in adipose tissue of treated mice. Overall, we show that adipose tissue is where most parasites accumulate throughout infection in mice and this may have important consequences for therapeutics and pathology.

5F Trypanosoma brucei infection accelerates the mouse circadian clock

Rijo-Ferreira, Filipa (Instituto de Medicina Molecular)*; Takahashi, Joseph S (HHMI UT Southwestern); Figueiredo, Luisa M (Instituto de Medicina Molecular)*

By living in a 24-hour world, organisms are subjected to daily environmental changes. Many organisms have evolved molecular mechanisms to anticipate such changes. In humans, the internal circadian clock regulates many physiological functions, including sleep/wake cycle and metabolism. Although the internal clock is controlled by the brain, all cells have an intrinsic clock. Patients with sleeping sickness show alterations of sleep/wake cycle, body temperature and endocrine secretion, which have led to the hypothesis that sleeping sickness may be a circadian rhythm disorder. We first infected mice with T. brucei and we recorded the circadian behavior using a running-wheel assay. We observed that infected animals run 2-fold less during the active phase and are 7-fold more active in the rest phase than healthy mice, confirming the changes in circadian behavior observed in patients. When we infected circadian reporter mice and measured the circadian parameters of several organs ex vivo, we observed that, although all organs have a robust circadian rhythm, those with higher parasite load have an internal clock that runs two hours faster than non-infected organs. These alterations were reproduced in vitro, when parasites were directly co-cultured with isolated fibroblasts, suggesting that parasites may have a direct effect on the host cell circadian clock. Finally we observed that expression of clock genes in vivo is significantly affected in peripheral tissues, especially in those with the highest parasite load. These results show that (i) T. brucei mouse infection reproduces circadian behavior changes observed in humans; (ii) T. brucei infection accelerates the mouse circadian rhythm by two hours; (iii) this effect may be partly caused by a direct interaction with the parasite.

5G Regulating the regulators: LmjPRMT7's role in trans-regulator expression and parasite virulence

Ferreira, Tiago R. (Ribeirao Preto Medical School, University of Sao Paulo);
Alves-Ferreira, Eliza V.C. (Ribeirao Preto Medical School, University of Sao Paulo); Defina, Tania P. (Ribeirao Preto Medical School, University of Sao Paulo); Smith, Deborah (Centre for Immunology and Infection, Department of Biology, University of York); Walrad, Pegine (Centre for Immunology and Infection, Department of Biology, University of York); Papadopoulou, Barbara (Research Center in Infectious Diseases, CHUL Research Center (CHUQ) Laval University); Cruz, Angela K. (Ribeirao Preto Medical School, University of Sao Paulo)

Arginine methyltransferases (PRMTs) catalyze the post-translational methylation of a wide spectrum of proteins in different cellular processes. Remarkably, RNA-binding proteins (RBPs) are major substrates of PRMTs and modification may result in altered function. The Leishmania major genome encodes five PRMT homologs, including PRMT7, which is only described in a restricted group of eukaryotes. We have found that both LmjPRMT7 expression and arginine monomethylation of proteins are tightly regulated during promastigote development; displaying minimal levels at the stationary growth phase and elevated LmjPRMT7 expression in procyclic promastigote and amastigote stage parasites. Nine RNA-binding proteins (RBPs) were found to co-immunoprecipitate with LmjPRMT7, indicating putative substrates. We expressed five myc-tagged putative targets in wild type and ∆lmjprmt7 null mutant parasites and assessed their methylation levels among samples by immunoblotting. The resulting data suggests that LmjPRMT7 methylates Alba20 protein in vivo and this modification leads to differential expression of this RBP. Considering LmjPRMT7 stage-specific expression, we evaluated the virulence of engineered transfectants. Remarkably, the ∆lmjprmt7 null mutant led to an increased virulence in mice, while LmjPRMT7-overexpressing parasites displayed attenuated virulence, both in vitro and in vivo. This work is the first to describe a possible role of Leishmania protein arginine methylation in the regulation of gene expression and to show a correlation between LmjPRMT7 levels and parasite virulence.

5H Association of Leishmaniavirus LRV1 with chemotherapeutic treatment failures of human leishmaniasis

Beverley, Stephen M. (Washington University School of Medicine)*; Adaui, Vanessa (Universidad Peruana Cayetano Heredia); Lye, Lon-Fye (Washington University School of Medicine); Akopyants, Natalia S. (Washington University School of Medicine); Zimic, Mirko (Universidad Peruana Cayetano Heredia); Llanos-Cuentas, Alejandro (Universidad Peruana Cayetano Heredia); Garcia, Lineth (Universidad Mayor de San Simon, Bolivia); Maes, Ilse (Institute of Tropical Medicine, Antwerp); De Doncker, Simonne (Institute of Tropical Medicine, Antwerp); Dobson, Deborah E. (Washington University School of Medicine); Arevalo, Jorge (Universidad Peruana Cayetano Heredia); Dujardin, Jean-Claude (Institute of Tropical Medicine, Antwerp)*

Cutaneous and mucosal leishmaniasis caused by Leishmania braziliensis is widespread in South America, and difficult to cure by current chemotherapy (primarily pentavalent antimonials). The factors contributing to clinical treatment failures are not well understood presently. Many isolates of L. braziliensis contain a double-stranded RNA virus termed Leishmaniavirus LRV1. LRV1 also occurs widely in L. guyanensis where association with increased pathology, metastasis, and parasite replication was found in murine models (Ives et al Science 2011). Here we probed the relationship of LRV1 to drug treatment success and disease in L. braziliensis. We studied a group of 97 patients from Peru and Bolivia, all showing cutaneous or mucosal leishmaniasis, and treated with Sb(V) (93) or Amphotericin B (4). In vitro cultures were established, the parasites were typed as L. braziliensis, and the presence of LRV1 was determined by RT-PCR with 'universal' LRV primers. The presence of LRV1 increased significantly the risk of treatment failure (odds ratio = 3·99, P = 0.04). There was no significant association between the presence of LRV1 and clinical presentation (diagnosis of CL vs. ML/MCL) at the time of parasite recovery, or with parasite Sb(V) resistance as assayed by treatment of macrophages infected in vitro. The mechanism by which the presence of LRV1 leads to a higher risk treatment failure is not known. LRV1 is correlated with resistance to oxidative stress which is postulated to be a significant part of SbV action (Hartley et al 2014 Trends Parasitol.). Alternatively (and not exclusively), potential LRV1-mediated increases in parasite numbers could overcome the often marginal efficacy of existing anti-leishmanial agents. Regardless, the association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of tegumentary disease caused by L. braziliensis. correspondence: beverley@wusm.wustl.edu

SESSION VI: Elisabetta Ullu Tribute

Shulamit Michaeli, Chair.

Tuesday 2:00pm

04/28/2015

6A The rich repertoire of snoRNAs and the identification of dual-function snoRNAs in Trypanosoma brucei

Michaeli, Shulamit (Bar-Ilan University)*; Chikne, Vaibhav (Bar-Ilan University); Doniger, Tirza (Bar-Ilan University); Eliaz, Dror (Bar-Ilan University); Semo, Oz (Bar-Ilan University); Cohen-Chalamish, Smadar (Bar-Ilan University); Tkacz, Itai Dov (Bar-Ilan University); Biswas, Viplop (Bar-Ilan University); Kolev, Nikolay G. (Yale university); Unger, Ron (Bar-Ilan University); Tschudi, Christian (Yale university)*

We identified the repertoire of small RNAs of procyclic- and bloodstream-form Trypanosoma brucei . Among the developmentally regulated small RNAs are the small nucleolar RNAs (snoRNA), which guide the two major base modifications, namely 2'- O -methylations (Nm) and pseudouridylation by C/D and H/ACA snoRNAs, respectively. Using RNA-seq of snoRNAs associated with C/D and H/ACA RNP proteins we identified the complete repertoire of these RNAs, which includes 80 molecules of each type in both T. brucei and L. major. The majority are predicted to guide modifications on rRNA. We applied a genome-wide approach to map the pseudouridines in the entire transcriptome of the parasite. All of the 80 predicted pseudouridines guided by H/ACA were verified on T. brucei rRNA. To identify the Small Cajal body (sca) RNAs, which in other eukaryotes guide modification on snRNAs, we identified the RNAs associated with a kinetoplastid-specific protein designated methyltransferase-associated protein (TbMTAP) (1), which its homologue binds scaRNAs in mammals. We then established a "deep walk" method to capture the sca-like RNAs via their cross-linking to snRNAs in vivo . Independently, we identified the same set of sca-like RNAs associated with the small RNA, TbsRNA-10 (2). We will present data demonstrating that many of the H/ACA RNAs have dual function and can guide modification on both rRNA and snRNAs. This novel mechanism may compensate for the presence of only a single hairpin H/ACA in trypanosomes compared to two hairpin H/ACA RNAs present in most eukaryotic H/ACA RNAs . 1. Zamudio J.R., et al., (2009). Mol. Cell Biol 29:1202-1211. 2. Michaeli S., et al., (2012) Nucleic Acids Res. 40: 1282-1298.

6B Quantitative Analysis of the Histone Acetylome and its Role in Histone Variant Deposition

Kraus, Amelie J. (Research Center for Infectious Diseases (ZINF), University of Würzburg); *ElBashir, Rasha (Rudolf-Virchow-Center, University of Würzburg); Vanselow, Jens T. (Rudolf-Virchow-Center, University of Würzburg); Wedel, Carolin (Research Center for Infectious Diseases (ZINF), University of Würzburg); Janzen, Christian J. (Theodor-Boveri-Institute, University of Würzburg); Förstner, Konrad U. (Research Center for Infectious Diseases (ZINF), University of Würzburg); Schlosser, Andreas (Rudolf-Virchow-Center, University of Würzburg); Siegel, T. Nicolai (Research Center for Infectious Diseases (ZINF), University of Würzburg)*

In eukaryotes, the dynamic alteration of chromatin is an important means for gene regulation. More open chromatin makes DNA accessible for the transcriptional machinery. Exchange of canonical histones with variant forms and post-translational modification of histones can induce chromatin reorganization. In many organisms, including Trypanosoma brucei , transcription start sites (TSSs) are enriched in acetylated histones and the histone variant H2A.Z. Despite growing knowledge how these marks mediate a more open chromatin, the mechanisms responsible for H2A.Z deposition are not yet clear. We established a method for the isolation of nucleosomes specifically from TSSs, which allowed us to elucidate the role of histone acetylation in H2A.Z deposition in T. brucei . Combined with a novel mass spectrometry-based approach, we could precisely quantify acetylation levels at individual lysines. Comparative analyses of TSS-nucleosomes and non-TSS nucleosomes enabled us to determine acetyl-marks enriched at TSSs. Using cell lines depleted for various acetyltransferases (HATs), we identified HAT2 to be solely responsible for TSS-nucleosome acetylation. Furthermore, to evaluate the effect of TSS-nucleosome acetylation on H2A.Z deposition, we performed more than 30 H2A.Z ChIP-Seq experiments after depletion or overexpression of different HATs and other chromatin factors. Our results indicate that HAT2 depletion leads to a loss in H2A.Z deposition, while its overexpression appears to cause unspecific H2A.Z incorporation throughout the genome. Taken together, the accurate quantification of histone acetylation allowed us to determine the complete trypanosome histone-acetylome and the enzymes responsible for individual acetyl marks. Moreover, our results reveal for the first time a strict dependence of H2A.Z deposition on histone acetylation in T. brucei and suggest an evolutionary conserved mechanism of histone variant deposition.

6C Codon usage is the major determinant of mRNA levels in Trypanosoma brucei

***de Freitas Nascimento, Janaina (University of Cambridge)**; Kelly, Steve (University of Oxford); Sunter, Jack (University of Cambridge); Carrington, Mark (University of Cambridge)*

In trypanosomes, most or all protein coding genes are constitutively transcribed and there is little evidence for selective regulation of expression by RNA polymerase II. Thus, regulation of mRNA levels is largely post-transcriptional. In this work we developed a novel codon usage metric called the 'gene expression codon adaptation index' (geCAI) that is predictive of both relative protein abundance and relative mRNA abundance with a coefficient of determination of 0.84 and 0.55 respectively. The predictive capacity of this novel scoring metric was tested using GFP reporter gene expression. 22 synonymous GFP mRNAs were expressed in procyclic cell lines. Protein expression and mRNA levels were modifiable over a ~40-fold range. The range and expression levels were similar to the measured mRNAs per haploid gene copy for the transcriptome and thus geCAI score is sufficient to account for 50% of the variation observed in mRNA copy number. GFP mRNAs with low geCAI scores decayed more rapidly than those with high scores suggesting translational efficiency is the mechanism that produces the differences in mRNA steady state levels. The translational efficiency model was tested by selectively blocking translation of individual GFP mRNAs by insertion of a hairpin in the 5'UTR, this equalized steady state levels to that of high geCAI score GFP mRNAs. In contrast, inclusion of a short upstream open reading frame in the 5'UTR greatly decreased translation of GFP and decreased steady state mRNA levels to that of a very low geCAI score GFP mRNA. Thus, geCAI is a good predictor of mRNA levels and efficiency of translation is a major determinant of mRNA levels.

6D Biased codons control mRNA and protein abundance in the African trypanosome

Jeacock, Laura (University of Dundee)*; Glover, Lucy; Hutchinson, Sebastian; Horn, David*

Cellular proteins, by necessity, differ in abundance over a wide range, from millions to only a few copies per cell and codon-usage bias is just one of a number of factors that can contribute to this differential. Trypanosoma brucei, presents an excellent model for studies on the impact of codon-bias because transcription is broadly uniform across the genome; T. brucei have no regulated RNA polymerase II promoters and transcription is constitutive and polycistronic. Here, we explore the contribution that codon-usage makes to differential mRNA and protein abundance in T. brucei. An experimental assessment of the impact of codon-usage confirmed our prediction, that protein abundance is increased by GC3 codons. This interpretation was supported by assays using synthetic luciferase and GFP-based reporters and indicated a capacity for control of relative protein abundance over several orders of magnitude by coding-sequence alone. Notably, mRNA abundance was also increased by GC3 codons. We next derived a simple formula (a = CAI - l) that predicts relative mRNA or protein abundance (a) based on Codon Adaptation Index and a penalty for gene length (l). Observed measures of abundance, from transcriptome and proteome data for thousands of distinct mRNAs and proteins, correlate with our predictions and this correspondence between CAI and protein abundance improved when we examined protein complexes. We suggest that GC3 codons increase the rate of mRNA translation and thereby also reduce mRNA turnover, and conclude that codon-usage is a post-transcriptional regulator of gene expression that has a major impact on mRNA and protein abundance in T. brucei.

6E The Functional Architecture of Trypanosoma brucei Editosomes Revealed by Cross-linking Mass Spectrometry and Random Mutagenesis

McDermott, Suzanne M. (Seattle Biomed)*; Luo, Jie (Institute of Systems Biology); Carnes, Jason (Seattle BioMed); Ranish, Jeff (Institute of Systems Biology); Stuart, Ken (Seattle BioMed)*

Uridine insertion and deletion RNA editing creates functional mitochondrial mRNAs and is catalyzed by three ~20S editosomes that differ in their endonuclease/partner protein pairs and cleavage specificity. Developmental regulation of editing between life cycle stages is associated with the switch between glycolysis and oxidative-phosphorylation. In order to better understand editosome architecture and the regulation of editing we used cross-linking/mass spectrometry (XL/MS), combined with a novel and widely applicable mutagenesis screen to obtain structural and functional data with amino acid level resolution. The spatial organization of proteins within purified editosomes was determined by XL/MS, which identified 355 inter-links between 74 protein pairs and 294 intra-links in 19 proteins. The cross-links are fully consistent with all current knowledge of editosome protein interactions and are validated by genetic and biochemical studies. Furthermore, the XL/MS cross-links provide the first evidence of specific interactions between the endonucleases and other editosome proteins. The results were used to create a highly detailed structural map of editosomes and to provide spatial constraints that we are using to guide integrative structural modeling of editosomes. We also developed a random mutagenesis screen, which was used in conjunction with site-directed mutagenesis to study the function of essential editosome proteins with no known catalytic functions. Mutations in 77% of KREPB5 amino acids identified essential residues within the RNase III-like and PUF domains and in regions that have no detectable homology to known motifs. We identified residues that are essential for bloodform survival but not procyclic form, and also mutations with a reciprocal effect. Hence editosome protein functions that differ between life cycle stages were identified in T. brucei.

6F Novel insights into RNP granules by employing the trypanosome's microtubule skeleton as a molecular sieve

Kramer, Susanne (University of Wuerzburg)*; Fritz, Melanie (University of Wuerzburg); Vanselow, Jens (University of Wuerzburg); Sauer, Nadja (University of Wuerzburg); Lamer, Stephanie (University of Wuerzburg); Goos, Carina (University of Wuerzburg); Siegel, Tim Nicolai (University of Wuerzburg); Subota, Ines (University of Wuerzburg); Schlosser, Andreas (University of Wuerzburg); Carrington, Mark (University of Cambridge)*

RNP granules, such as P-bodies and stress granules, are ribonucleoprotein assemblies that regulate the post-transcriptional fate of mRNAs in all eukaryotes. Their exact function remains poorly understood, one reason for this is that RNP granule purification has not yet been achieved. We have exploited a unique feature of trypanosomes to prepare a cellular fraction highly enriched in starvation stress granules. First, granules remain trapped within the cage-like, subpellicular microtubule array of the trypanosome cytoskeleton while soluble proteins are washed away. Second, the microtubules are depolymerized and the granules are released. RNA sequencing combined with single mRNA FISH revealed that mRNA localization to granules positively correlates with mRNA size and negatively with mRNA abundance. The most prominent examples for granule-excluded mRNAs are the short and highly abundant mRNAs encoding ribosomal proteins. By mass spectrometry we have identified 463 stress granule candidate proteins. For 18 proteins we have validated the localization to granules, including two phosphatases, one methyltransferase and two proteins with a function in trypanosome life-cycle regulation. Our protocol is rapid, granule dissociation and mRNA degradation are minimized and high throughput experiments are possible. It should be applicable to all protozoa with a tight microtubules cytoskeleton, for example Apicomplexa. Given the conservation of RNP granules throughout the eukaryotic kingdom, results are likely transferrable to other eukaryotes. This is the first identification of an RNP granule's mRNA content and our data are consistent with i) mRNA localization to granules being based on the amount of binding platforms for RNA binding proteins that mediate granule aggregation and ii) a function of the granules in mRNA decay.

6G Identification of the First Direct Interaction between the Surface of Trypanosoma brucei and the Innate Immune System: a Factor H Receptor

***Macleod, Olivia JS (University of Cambridge)**; Carrington, Mark (University of Cambridge)*

Trypanosoma brucei interacts with its hosts through proteins expressed on the external face of the plasma membrane. The transferrin receptor (TfR) is required for the uptake of transferrin from the host, but its structure is unknown. The haptoglobin haemoglobin receptor (HpHbR) takes up host HpHb, as well as primate-specific innate immunity factor TLF1. The structure of the T. b. brucei HpHbR has recently been shown to have a kinked structure, allowing for ligand uptake within the context of the VSG coat. The TfR and HpHbR are thought to be restricted to the flagellar pocket by an unknown mechanism. What other molecular interactions are there between the trypanosome surface and the host? Here, a GPI-anchored surface receptor has been identified that binds host complement factor H (CFH). The role of CFH is regulation of the innate immune system by preventing the alternative complement pathway from progressing and therefore stopping cell lysis. The CFH receptor is expressed in both bloodstream and procyclic forms in culture. However, expression in bloodstream forms is suppressed by passage in medium containing heat-inactivated serum, but can be reactivated by transfer to medium containing normal serum. The evolution of a CFH receptor is an example of convergent evolution, as a range of other pathogens have distinct CFH receptors. These observations are the first identification of a direct interaction between immune system effectors and T. brucei, and represent a further mechanism of immune evasion in the mammalian blood, both in situ and in a blood meal.

6H Leishmania IFT140 mutants show normal viability but are aflagellate: a tool for the study of flagellar function through the infectious cycle

F*owlkes, Tiffanie* *(Washington University-St. Louis); Beverley, Stephen (Washington University-St. Louis)*

The Leishmania flagellum is dramatically remodeled during development; the promastigote flagellum is long while the amastigote flagellum barely protrudes past the surface. In promastigotes, the flagellum functions in motility and potentially in cell morphogenesis, division, environmental signaling, and maintenance of the flagellar pocket (FP). Amastigotes may share these 'housekeeping' functions, and the flagellum is also proposed as a conduit for transfer of parasite factors into the macrophage. In the long flagellum of trypanosomes, flagellar assembly and cell viability require intraflagellar transport (IFT), but this may differ in the short amastigote flagellum. To test this, we targeted a core retrograde pathway gene IFT140 in L. donovani strain Bob. We used a plasmid segregation test of an episomal IFT140 in a chromosomal Δift140 knockout (Murta et al Mol. Micro. 2009). Unexpectedly, we readily obtained viable knockout promastigotes which grew normally, lacked an external flagellum, and were immotile. EM analysis showed no axoneme assembly within the FP. The FP contained many vesicles but was otherwise normal, including maintaining the neck region through the collar. Preliminary data showed small secretion effects, and Δift140 lacked detectable levels of the major components of the paraflagellar rod. Complementation with episomal IFT140 restored all mutant phenotypes to WT. Thus Δift140 is the first example of a healthy aflagellate promastigote; its remarkably normal phenotype suggests that unlike trypanosomes, the Leishmania promastigote flagellum is not required for cell division, secretion, or FP morphology. The ability of LdBob to differentiate to amastigotes in vitro and infect mammalian hosts allows us to probe the requirement for IFT function in the short flagellum of amastigotes and its effects on parasite virulence.

SESSION VII: Cell Biology II - Mark Field, Chair. Wednesday 8:45am

04/29/2015

7A Complexities in coordinating morphogenesis - proximal end assembly of the flagellum attachment zone contrasts to the flagellum's distal assembly

Sunter, Jack (University of Oxford)*; Varga, Vladimir (University of Oxford); Dean, Samuel (University of Oxford); Gull, Keith (University of Oxford)*

A defining morphological feature of Trypanosoma brucei is the lateral attachment of its single flagellum to the cell body, mediated by a cytoskeletal structure called the flagellum attachment zone (FAZ). The FAZ is large and complex, encompassing three major regions: the fibres that extend from the axoneme and paraflagellar rod to the flagellar membrane; the flagellar and cell body membranes including the macula adherens that maintain the attachment between these two membranes; and the FAZ filament and associated microtubule quartet within the cell body and the connections from them to the cell body membrane. The FAZ is a key morphogenetic structure and modulation of FAZ assembly leads to diverse phenotypes including dramatic changes in cell shape and flagellum detachment. Despite the complexity of this structure few molecular components are known, limiting our ability to understand the form, function and assembly of this supramolecular structure. We have used a combination of proteomics and bioinformatics to identify eight new FAZ proteins. These new FAZ proteins, representative of the cell body and membrane regions, had distinct lateral localisations within the FAZ, allowing definition of specific sub-domains within this structure. Using inducible expression of FAZ proteins tagged with eYFP we demonstrate that the site of FAZ assembly is close to the flagellar pocket at the proximal end of the FAZ. This contrasts with the flagellum, which is assembled at its distal end; these two interconnected cytoskeletal structures hence have distinct spatially separated assembly sites. This challenging result has many implications for understanding the process of cell morphogenesis and interpreting mutant phenotypes.

7B Components and function of the basal body transition zone in Trypanosoma brucei

Dean, Samuel (University of Oxford)*; Gull, Keith (University of Oxford)*

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, 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 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. Despite this, few proteins have been identified that localise to the transition zone in trypanosomes, and the proteins that make up the characteristic structures of the transition zone (such as the Y shaped linkers that link the axoneme to the cell membrane, and transitional fibers that act as docking sites for IFT vesicles) are largely uncharacterised in any organism. We have used a novel proteomic strategy to purify the cytoskeletal architecture encompassing the transition zone. To date, we have identified >50 proteins that localise to the transition zone and associated structures. These include novel components of the Y-shaped linkers, transitional fibers and flagellar pocket neck as well as ciliopathy complexes. Analysis of these proteins show that the transition zone is a remarkably stable structure that exhibits little protein turnover throughout the cell cycle. RNAi depletion of these proteins causes a range of phenotypes, ranging from no cytological peturbance to loss of the central pair of microtubules and complete loss of the flagellum. This highlights the importance and variety of functions of this flagellum sub-compartment.

7C The Trypanosoma brucei transmembrane surface protein TbGPCR-L1 induces stumpy formation through the quorum sensing signalling pathway

Rojas, Federico (University of EdinburghCentre for Immunity, Infection and Evolution, Institute for Immunology and infection Research, School of Biological Sciences, A)*; Thompson, Joanne (Centre for Immunity, Infection and Evolution, Institute for Immunology and infection Research, School of Biological Sciences, Ashworth Laboratories, U); Matthews, Keith R (Centre for Immunity, Infection and Evolution, Institute for Immunology and infection Research, School of Biological Sciences, Ashworth Laboratories, U)*

G protein-coupled receptors are seven transmembrane domain (TM) proteins that transduce signals through their interactions with extracellular ligands and G protein-dependent and -independent signaling cascades, allowing cells to respond to changes within their environment. Although trypanosomes are conventionally described as lacking GPCRs, we have identified a T. brucei protein (TbGPCR-like 1; TbGPCR-L1) related to GPR89 family members that have been implicated in abscisic acid signaling in plants and Golgi acidification in mammals. The T. brucei protein has 8-9 predicted TM, a 90 amino acid intracellular loop and localizes to the surface membrane. Western blot and immunofluorescence analysis shows that in pleomorphic trypanosomes (i.e. capable of generating slender and stumpy forms), TbGPCR-L1 is expressed on slender forms but levels decrease as cells differentiate into intermediate and stumpy forms. In pleomorphic parasites, in contrast to monomorphic cells, inducible overexpression of TbGPCR-L1 protein drives premature differentiation in vitro and in vivo, generating stumpy cells at lower cell density compared to uninduced or the parental cell line. Upon overexpression of TbGPCR-L1 in an RNAi background targetting RBP7 or PP1 (two proteins previously shown to be required for normal quorum sensing) cells do not differentiate into stumpy cells, suggesting that TbGPR89-mediated signalling acts upstream of these genes but on the same pathway. When the intracellular loop of the protein (containing several phosphorylation sites and MAPK binding motifs) is replaced with a 6HA tag and overexpressed, cells do not differentiate prematurely into stumpy forms, highlighting that this region is necessary for its signalling capabilities. Our experiments indicate that TbGPR89-L1 might be expressed on slender forms as a sensor of an external stimulus capable initiating stumpy formation.

7D Functional characterization of small proteins in Trypanosoma brucei

Ericson, Megan (Yale University)*; Obado, Samson (Rockefeller University); Kobayashi, Junya (Nagoya University); Gould, Matthew (University of Edinburgh); Schnaufer, Achim (University of Edinburgh); Matsuura, Yoshiyuki (Nagoya University); Rout, Michael (Rockefeller University); Ullu, Elisabetta (Yale University); Tschudi, Christian (Yale University)*

The identification of all expressed proteins remains a major challenge, especially those less than 100 amino acids in length. Our previous analysis of small proteins in Trypanosoma brucei exposed 7 to be essential in the procyclic life cycle stage, with 2 of the 7 also required for bloodstream-form viability. One, Tb11.NT29, a protein of 62 amino acids, localized to the flagellum. RNAi-induced knockdown of Tb11.NT29 resulted in flagellar detachment, arrest in cell cycle progression after kinetoplast duplication, and perturbation in basal body structure. The other small protein essential in both life cycle stages, Tb10NT87, is 64 aa in length and localized to the matrix of the mitochondria. Tb10NT87 appeared to be in a complex of approximately 200 kDa that also included TbKap123, a karyopherin-like protein. RNAi against Tb10NT87 resulted in an accumulation of cells with smaller kDNA, a decrease in the steady-state levels of mitochondrial transcripts and the accumulation of tRNA halves in the mitochondrion. TbKap123, also essential in both procyclic and bloodstream life-cycle stages, was localized in the mitochondria, nucleus, and cytoplasm. Interestingly, RNAi against TbKap123 did not affect kDNA size or mitochondrial transcript abundance. RNAi of TbKap123 did, however, result in a reduction in the steady-state levels of certain mitochondrial proteins, implying a role in mitochondrial protein import. To determine whether the primary role of Tb10NT87 or TbKap123 was kDNA maintenance, a dyskinetoplastic cell line was generated in the bloodstream stage of T. brucei. Following RNAi, Tb10NT87 was no longer essential, inferring a role in kDNA maintenance, but TbKap123 was still required for parasite viability, indicating that this protein plays multiple roles in the cell.

7E Differential targeting to flagellum membrane sub-domains influences receptor adenylate cyclase function in Trypanosoma brucei social behavior

Saada, Edwin A. (UCLA)*; Hill, Kent (UCLA)*

The kinetoplastid flagellum is recognized for its motility functions, but increasing evidence indicates it also functions in signaling and that flagellar membrane proteins present an important host-parasite interface. In proteomic analyses of T. brucei flagella, we identified subsets of surface-exposed receptor adenylate cyclases (ACs). ACs are postulated to function in cAMP signaling by directly coupling a large, extracellular ligand-binding domain to an intracellular adenylate cyclase catalytic domain. Immunofluorescence localization of procyclic-specific ACs revealed distinct localizations for different AC paralogs. One group localized along the length of the flagellum, while others specifically localized to the flagellum tip, indicating specialization of flagellum membrane subdomains. Functional analyses reveal that cAMP regulates trypanosomal social motility via flagellar ACs together with cAMP-specific phosphodiesterase. To ask whether AC flagellar subdomain localization impacts AC function in social motility, we employed truncation mutants and chimeric proteins to define flagellar- and tip-targeting sequences. We identified a 45 amino acid segment within the intracellular domain that is necessary for flagellum membrane targeting and further defined a nine amino acid sequence within this domain that directs targeting to the flagellar tip. Re-direction of a flagellum-length AC to the flagellum tip rescued the social motility phenotype of flagellum-tip AC knockdown, implicating flagellum tip-specific cAMP signaling as a key regulator of social motility. Studies on differentially localized flagellar receptors advance understanding of principles that govern protein targeting to flagellum subdomains and provide insight into T. brucei signaling mechanisms, both of which are poorly understood but fundamentally important features of trypanosome biology.

7F VSG identity and structural integrity determine growth rate of bloodstream form Trypanosoma brucei

Schwede, Angela (University of Cambridge)*; Jones, Nicola (University of Wuerzburg); Engstler, Markus (University of Wuerzburg); Carrington, Mark (University of Cambridge)*

Trypanosome antigenic variation is based on extreme sequence variation in VSGs. How does the trypanosome accommodate different rates of VSG synthesis, folding and maturation? One possibility is that the VSG is always synthesised in excess so that growth is never limited by the identity of the VSG. Alternatively, the trypanosome could adapt growth rate to match the rate of VSG synthesis or VSG coat integrity. Here, we show that growth rate is determined by the identity of the VSG. The growth rate of a cell line expressing a 'slow' VSG was increased by ectopic expression of a 'fast' VSG transgene. In contrast, growth was dramatically slowed by the ectopic expression of VSG transgenes containing structural mutants. This phenotype was stable for several weeks without loss of viability and was fully reversible, reversion to normal growth resumed on inactivation of the transgene. Further, growth rate could be altered within hours using tetracycline inducible VSG transgenes. These results show that the trypanosome can adapt its growth rate in response to the VSG. A comparison of slow and fast growth transcriptomes was used to identify possible effectors of growth rate. Three of the findings are: (i) the slow growth transcriptome is different to the 'stumpy' transcriptome. (ii) One possible effector, PIP39, was validated and expression in bloodstream forms is inversely proportional to growth rate. Overexpression of PIP39 is sufficient to slow growth. (iii) Amongst the 47 most regulated mRNAs, 14 are involved in glucose metabolism. The up regulation of glycolysis in fast growing trypanosomes resembles the Warburg effect observed in cancer cells.

7G The role of VSG in T. brucei evasion from macrophage phagocytosis

Cheung, Jackie (Imperial College London)*; Wand, Nadina (Imperial College London); Wheeler, Richard (The Sir William Dunn School of Pathology, University of Oxford); Rudenko, Gloria (Imperial College London)*

The Variant Surface Glycoprotein (VSG) coat of bloodstream form Trypanosoma brucei is essential for evasion of the host immune system. During infection, there is rapid VSG recycling and antibodies binding the expressed VSG variant are endocytosed and degraded. Previously, we have shown that blocking the synthesis of VSG using tetracycline inducible RNAi triggers a precytokinesis cell cycle arrest and a global block in translation. Here, we investigated the role of VSG in the evasion of T. brucei from phagocytosis by macrophages. After the induction of VSG RNAi, we observed a 3.4-fold increase in trypanosome phagocytosis by macrophages in the presence of anti-VSG antibodies. Furthermore, cell cycle analysis revealed that internalisation of anti-VSG antibodies occurs at similar rates throughout the cell cycle in wild type cells. However, after blocking VSG synthesis, all cells clear anti-VSG antibodies at a significantly reduced rate, but this effect is particularly pronounced in G2/M stage cells. Although there is a significant reduction in anti-VSG antibody clearance after the induction of VSG RNAi, the rates of internalisation of biotinylated VSG as well as fluid-phase endocytosis or endocytosis of tomato-lectin remain unaffected. Interestingly, blocking the synthesis of VSG causes T. brucei to swim in a more directional manner (possibly due to the restriction operating on the surface coat) but has no significant impact on the swim speed. Our data indicate that continuous synthesis of VSG is crucial for the effective clearance of anti-VSG antibodies by bloodstream form T. brucei . Reduction in the rate of anti-VSG antibody removal results in increased phagocytosis by macrophages, highlighting the crucial role of VSG recycling in immune evasion during chronic infections.

POSTERS: Session A
Sunday 7:00pm

04/26/2015

69 What do kinetoplastids need a kinetoplast for? Life cycle progression of Trypanosoma brucei in the presence and absence of mitochondrial DNA

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We recently showed that mutations in the nuclearly encoded ? subunit of the mitochondrial FoF1-ATPase (such as L262P) negate the requirement for mitochondrial DNA (kDNA) in long slender bloodstream form (LS) Trypanosoma brucei (Dean et al., 2013). We now investigate two questions: (1) What is the molecular mechanism of compensation for kDNA loss? (2) Are kDNA and a functional FoF1-ATPase required for life cycle progression? The fundamental differences between energy metabolism in procyclic insect form (PF) and LS T. brucei involve a switch in FoF1-ATPase directionality. In PF, oxidative phosphorylation requires the enzyme to generate ATP. In LS, the enzyme uses ATP from glycolysis to drive proton pumping to maintain the essential mitochondrial membrane potential. Fo-ATPase subunit 6 is critical for proton translocation in either direction and is kDNA-encoded. Using biochemical analyses we now confirm that the L262P? mutation uncouples the F1 and Fo parts of the enzyme, and show that an uncoupled enzyme and intact Fo appear to be incompatible: several independent clones show loss of fully edited subunit 6 mRNA and concomitant loss of Fo. We will discuss how ? mutation and kDNA loss, respectively, affect structure/function of the FoF1-ATPase. Differentiation studies with pleomorphic T. brucei expressing L262P? in a kDNA+ or kDNA0 background demonstrate that both types of cell can differentiate to insect-transmissible stumpy forms. However, tsetse fly infections show kDNA is indispensable for progression to the PF form. Unexpectedly, heterozygous L262P? kDNA+ cells can form animal-transmissible metacyclics in the salivary glands. Since these cells are resistant to multiple drugs in vitro (Gould and Schnaufer, 2014), the L262P? mutation provides a potential mechanism for the spread of resistance.

70 Ribosome profiling reveals translation control as a key mechanism generating differential gene expression in Trypanosoma cruzi

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Due to the absence of transcription initiation regulation of protein coding genes transcribed by RNA polymerase II, posttranscriptional regulation is responsible for the majority of gene expression changes in trypanosomatids. Therefore, cataloging the abundance of mRNAs (transcriptome) and the level of their translation (translatome) is a key step to understand control of expression in these organisms. Here we assess the extent of regulation of transcription and translation in the parasite Trypanosoma cruzi, in both the non-infective (epimastigote) and infective (metacyclic trypomastigote) life stages using RNA-seq and ribosome profiling. The observed steady state transcript levels support constitutive transcription and maturation implying the existence of distinctive posttranscriptional regulatory mechanisms controlling expression levels at those stages. Meanwhile, down regulation of a large proportion of the translatome indicates a key role of translation control in differentiation into the infective form. The previously described proteomic data correlate better with the translatomes than with the transcriptomes and translational efficiency analysis shows a wide dynamic range, reinforcing the importance of translatability as a regulatory step. Translation efficiencies for protein families like ribosomal components are diminished while translation of the transialidase virulence factors is up regulated in the quiescent infective metacyclic trypomastigote stage. A large subset of genes is modulated at the translation level in two different stages of Trypanosoma cruzi life cycle. Translation up regulation of virulence factors and downregulation of ribosomal proteins indicates different degrees of control operating to prepare the parasite for an infective life form. Taking together our results show that translational regulation, in addition to regulation of steady state level of mRNA, is a major factor playing a role during the parasite differentiation.

71 Development of an inducible system for Leishmania gene deletion; application to the cell cycle protein kinase CRK3

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Despite the development of a number of reverse genetic techniques to probe gene function in Leishmania , a conditional system for regulating the expression of genes essential for parasite survival is lacking. To address this, we have implemented an inducible gene knockout system based on a dimerised Cre recombinase (diCre) to target the gene encoding an essential cdc-2 related kinase (CRK3) and elucidate its role in the cell cycle of L. mexicana . Cre recombinase mediates the excision of DNA sequences flanked by 34bp loxP sites (‘floxed’). diCre is encoded as two separate subunits each linked to rapamycin binding domains (FRB and FKBP12); therefore recombinase activity is induced upon rapamycin treatment (PMID: 14576331). Our method involves replacing both CRK3 alleles with a ‘floxed’ CRK3 open reading frame and the diCre coding sequence through promastigote transfection and homologous recombination. Induction of diCre through rapamycin treatment of promastigotes results in highly efficient deletion of CRK3 and a distinct growth arrest phenotype corresponding to a block in G2/M. Induced loss of CRK3 can be complemented by expression of a CRK3 transgene but not by expression of an inactive site (T178E) CRK3 mutant, showing that protein kinase activity is crucial for CRK3 function. Significantly, inducible deletion of CRK3 also occurs in lesion-derived amastigotes, resulting in a similar growth defect and cell cycle arrest, demonstrating an essential role in the amastigote cell cycle. This study represents an important step forward in the study of essential gene function through highly efficient inducible gene deletion in both life cycle stages of Leishmania . The application of the technique to the study of Leishmania biology and host-parasite interactions will be discussed.

72 Keeping an open chromatin in the active VSG expression site is associated to uncommitted switching

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Trypanosoma brucei evades the host immune system by periodically changing its cell surface Variant Surface Glycoproteins (VSGs) through antigenic variation. This process relies on monoallelic transcription by RNA polymerase I of a single VSG gene from one of the 15 subtelomeric Bloodstream Expression Sites (BES). It was previously shown that the chromatin of the highly transcribed active BES is depleted of nucleosomes and enriched in TDP1, a high mobility group box protein. However, it remains unclear how chromatin and transcription are inter-regulated to control monoallelic expression. Using a reporter cell-line that allows the inducible generation of VSG switchers, we observed that chromatin of the active BES is maintained accessible for at least eight hours after transcription has been blocked, indicating that the nucleosome-depleted conformation of the active BES is independent of transcription. We showed that maintenance of such accessible chromatin structure is independent of cell-cycle, but dependent of TDP1. For eight hours post-silencing induction, we found that cells were not committed to switching and could “go-back” and re-transcribe the initially active BES. Using a reporter gene in a silent BES, we observed that silent BES are transcriptionally probed at low levels while chromatin of active BES remains open. We propose that during VSG expression site switching, chromatin of the active BES is maintained in an accessible conformation by TDP1 for a short period, allowing cells to probe silent BESs before committing irreversibly to a new BES.

73 Multiple protein kinases provide diverse DNA repair-associated functions in Trypanosoma brucei.

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In eukaryotic cells, it is essential to efficiently and effectively repair damage to the genome in order to maintain cell viability. In all eukaryotes proteins kinases (PKs) contribute to repair, acting to prevent or limit cell cycle progression under damage stress, to co-ordinate the activity of repair proteins and to restart cell growth when damage is resolved. Trypanosoma brucei possess approximately 190 PKs, many of which remain unstudied and none of which have been shown to act as DNA repair PKs. A genome wide RIT-seq screen was performed, comparing growth in the presence and absence of methyl methanesulphonate (MMS), which generates widespread genome damage. Eight PKs were revealed that showed >2 fold loss of read mapping after RNAi in the presence of MMS relative to absence. Four of these PKs, none of which have been shown to act in repair in any organism, were validated by growth in the presence and absence of MMS, cell cycle analysis, as well as by measuring levels of phosphorylated histone H2A. In addition, a tousled-like kinase and ATR, two known eukaryotic repair PKs, were shown to mediate the T. brucei response to MMS damage, though both are essential, limiting their detection in the RITseq screen. Perhaps surprisingly, ATM shows no evidence for a role in MMS repair. Epitope tagging of the novel repair PKs reveals both nuclear and non-nuclear, region-specific subcellular localisation, indicating diverse functions, some of which appear to link repair or damage sensing with downstream cell division processes.

74 Basal body connector protein (BBCP), a basal body-specific protein interlinking with pro-basal body in Trypanosoma brucei

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Cytokinesis and duplication of centrioles are two well orchestrated processes in most metazoans but not in many other eukaryotes, such as Trypanosoma brucei . Revealing the basal body (BB) disengagement machinery in this eukaryotic organism would shed light on this important organelle composition and provide hits in the centriole evolution scenery. In this study we used proteomic approaches to identify novel BB protein in T. brucei BB connector protein (BBCP). Bioinformatics analysis predicts BBCP to be a long coiled-coil protein and conserved strictly in kinetoplastida. By YFP tagging, BBCP is found to localize between BB and pro-BB through out the cell cycle. RNAi against BBCP showed it to be an essential protein for cell proliferation and kinetoplast inheritance. BBCP depletion resulted in splitting of BB and pro-BB and mislocalization of pro-BB, which suggests that BBCP is important for the physical connection of BB/pro-BB. Transmission electron microscopy showed the mislocalization affects pBB's docking and flagellum extension but not maturation. The identification of BBCP not only enhances the knowledge of BBs/centrioles assembly and organization, but also provides a tool to study the maturation of pBB.

75 Defining the mode of action of anti-trypanosomatid compounds: A next generation platform

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The development of new drugs for the treatment of kinetoplastid diseases remains a challenge, hampered by few validated targets and insufficient understanding of basic kinetoplastid biology. These, and other, challenges have led to drug discovery programs that exploit phenotypic screening of compound collections against parasites in culture. Subsequent chemical optimization of these phenotypic actives can become challenging particularly where pharmacokinetic or selectivity issues arise. Circumvention of these issues would be greatly facilitated by knowledge of the mode of action (MoA) and the specific targets of phenotypic cell-actives. To address these issues we have established a multiplex platform for the determination of MoA. We have identified several hundred anti-kinetoplastid compounds through phenotypic screening of libraries, providing well-validated cell-active chemical matter encompassing a diverse range of chemical scaffolds. The diversity of the cell-actives suggests that multiple undefined MoAs are in play. Our MoA-determination platform incorporates a battery of chemical, biological and genetic tools, and the orthogonal basis of the platform provides particular strength in determination of the interactions between actives and the parasite. This should yield high value insights into MoA, also potentially defining mechanisms of resistance, and molecular targets. The utility of this approach will be discussed in terms of developing compounds as potential therapies and also as well characterised tool compounds for the dissection of kinetoplastid biology.

76 Cutaneous and mucosal Leishmania braziliensis isolates from the same patient show differential gene expression and infection profile

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Leishmania braziliensis is a major etiological agent of cutaneous leishmaniasis (CL) in Brazil and less than ten percent of patients with CL develop mucosal leishmaniasis. To identify parasite factors that contribute to the differential clinical manifestations in humans, we investigated possible phenotypic modifications between parasites from mucosal (LbrM) and cutaneous (LbrC) sites from the same patient. These isolates showed no major genomic divergences as indicated by molecular karyotype and genomic sequencing (NGS) and lacked Leishmania RNA virus (LRV). The cutaneous and mucosae isolated parasites led to distinct pathogenesis in Balb/c in vivo infection. Mice infected with LbrC showed larger lesions and higher parasite load than mice infected with LbrM. LbrC-infected mice cells displayed a predominant Th1 pattern after stimulation of L. braziliensis SLA in vitro while LbrM infection induced a Th2 pattern. In addition, we submitted these isolates to metabolomic and proteomic comparison. Metabolomic analysis revealed significantly increased levels of 16 metabolites in LbrC parasites and 38 metabolites in LbrM. Data suggest that differences between LbrC and LbrM tropism involves the differential production of metabolites related to inflammation and chemotaxis. A proteome comparative analysis revealed, among other genes, the overexpression of LbrPGF2S (prostaglandin f2-alpha synthase) and HSP70 in cutaneous isolates. Overexpression of LbrPGF2S in LbrC and LbrM promastigotes triggered an increment of infected macrophages and the number of amastigotes per cells 24-48h post-infection. Therefore, our results suggest that the inoculated population of parasites may contain subpopulation of cells with a divergent expressed genome that lead to distinct clinical manifestations and that LbrPGF2S protein is a possible candidate to contribute to the virulence profile of the parasite in the mammalian host.

77 KHARON1 Mediates Trafficking of Flagellar Membrane Proteins in African Trypanosomes and is Essential for Parasite Viability

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Flagellar membrane proteins play critical roles in kinetoplastid parasites including sensing of the extracellular environment, but mechanisms for selectively targeting such proteins to the flagellar membrane are obscure. We have identified a protein in Trypanosoma brucei , KHARON1, which is essential for flagellar targeting of a calcium channel and a calcium ATPase and potentially for a cohort of flagellar membrane proteins. RNAi knockdown of Kharon1 mRNA is lethal to both bloodstream and procyclic trypanosomes and results in formation of multinucleated 'monster' parasites that fail to undergo cytokinesis. KHARON1 localizes to the base of the flagellar axoneme, where it presumably mediates trafficking of proteins from the flagellar pocket into the flagellar membrane, but it is also present on the subpellicular microtubules and on the mitotic spindle where it likely carries out other cellular functions. KHARON1 appears to be part of one or more large multiprotein complexes, and some protein subunits may be selectively associated with the complex at distinct subcellular sites. Ongoing studies are probing the cohort of proteins that require KHARON1 for flagellar targeting, the identify of other KHARON Complex subunits, and the biological functions of the KHARON Complexes at the three distinct subcellular locations.

78 Characterization of the translationally controlled tumor protein in Trypanosoma brucei (TbTCTP)

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The translationally controlled tumor protein (TCTP) is a highly conserved protein expressed ubiquitously from single celled protozoa to plants and mammals. The protein seems to be involved in a wide range of biological functions such as protection against cellular stress, inhibition of apoptosis, cell cycle progression, growth and development etc.. However, a clear function and mechanism describing TCTP's physiological role is still lacking. In trypanosomes the ortholog of TCTP (TbTCTP) encodes for a 19 kDa hydrophilic protein with a completely unknown and not described function. RNAi knock down of TbTCTP in bloodstream trypanosomes led to cell cycle arrest prior to cytokinesis. The HA tagged protein localizes mainly to the cytosol with a pattern resembling viral particles, similar to what is described in other organisms. Based on a number of experiments we can show the protein to be involved in protection against heat shock stress. While in yeast TCTP changes localization following the stress conditions, in trypanosomes the tagged protein remains in the cytoplasm. Most kinetoplastid genomes reveal two paralogs of TCTP, which have almost identical ORFs but differ in the length and base composition of their 3'UTRs. Interestingly the two paralogs are differentially expressed during the life cycle of the parasite.

79 Structural basis for ligand and innate immunity factor uptake by the trypanosome haptoglobin-haemoglobin receptor

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The haptoglobin-haemoglobin receptor (HpHbR) of African trypanosomes allows acquisition of haem, whilst also being exploited by the host as an uptake route for trypanolytic factor-1 (TLF1), a mediator of innate immunity against trypanosome infection. We have used X-ray crystallography to determine the structure of HpHbR from Trypanosoma brucei in complex with human haptoglobin-haemoglobin (HpHb). This revealed an elongated binding site on the receptor, contacting both haptoglobin and haemoglobin, showing how the receptor selectively binds HpHb over the individual components. This binding mode will be conserved in trypanolytic factors, as polymorphisms in haptoglobin-related protein, a component of TLF1, are not in the receptor-binding interface. Lateral mobility of the glycosylphosphatidylinositol-anchored HpHbR and a ~50° kink in the receptor allow two receptors to simultaneously bind one HpHb dimer, which will increase the efficiency of ligand binding to cells. This study therefore reveals the molecular basis for ligand and innate immunity factor uptake by trypanosomes, and identifies adaptations that allow efficient ligand uptake in the context of the complex trypanosome cell surface.

80 Molecular characterization of 5S ribosomal RNA genes in Leishmania major

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Eukaryotic 5S ribosomal RNA (rRNA) is synthesized by RNA Polymerase III (Pol III), whereas other rRNA molecules are transcribed by Pol I. 5S rRNA is an essential component of the large ribosomal subunit, and it seems to regulate the relationships between the different ribosomal functional sites. Most organisms contain ~ 100 rRNA genes organized into tandem arrays. However, the L. major genome contains only 11 5S rRNA genes, which are interspersed and associated with other Pol III-transcribed genes. All these copies contain the putative internal promoter regions: boxes A and C and the intermediate element. These regions are necessary in other eukaryotes for the binding of transcription factors TFIIIA, TFIIIC and TFIIIB, which allow Pol III transcription. Interestingly, neither TFIIIA nor TFIIIC has been identified in trypanosomatids. By 5´-RACE experiments we showed that transcription of the 5S rRNA genes starts at a G that corresponds to the first nucleotide of the genes. In contrast, 3´-RACE assays demonstrated that Pol III transcription ends in a T-residue tract located downstream of all the genes. Electrophoretic mobility shift assays (EMSA) showed the specific union of a low-weight protein to the 5S rRNA gene. By Southwestern assays we found that this protein is about 32 kDa. It remains to be tested if this protein is the orthologue of TFIIIA in Leishmania . Analyses of the secondary and tridimensional structures of the 5S rRNA from L. major showed that both structures are highly conserved in relation to other eukaryotes. Preliminary combined Immunofluorescence and DNA FISH analysis suggest that 5S rRNA genes are located near the nucleolar periphery.

81 Characterization of the small Tim homologues in Trypanosoma brucei

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The small Tim (translocase of the mitochondrial inner membrane) proteins are mitochondrial intermembrane space (IMS) proteins that function as chaperones during the translocation of the nuclear-encoded mitochondrial polytopic membrane proteins through the IMS. Trypanosoma brucei, a protozoan parasite that is the causative agent of human African trypanosomiasis, possesses a single mitochondrion that requires hundreds of polytopic membrane proteins for its function. In contrast to the five small Tims (Tim9, Tim10, Tim12, Tim8, and Tim13) found in yeast and humans, T. brucei has only three small Tim proteins. Two of the small TbTim homologues show similarity to Tim9 and Tim10 (TbTim9 and TbTim10). The third small Tim homologue contains signatures of both Tim8 and Tim13, referred here as TbTim13/8. We found that the small TbTim homologues are expressed in T. brucei and localized in the parasite mitochondrion as soluble proteins. Knockdown of TbTim9 or TbTim10 reduced cell growth, whereas knockdown of TbTim13/8 ceased cell growth. Downregulation of the small TbTims reduced the steady-state level of the mitochondrial ADP/ATP carrier (TbAAC), the voltage-dependent anion channel (VDAC) of the outer membrane, and the inner membrane translocase TbTim17. Interestingly, TbTim17 levels were rapidly depleted upon RNAi induction, as compared to TbAAC or VDAC. Blue-Native gel electrophoresis revealed that the dimerization of TbAAC, which is the initial step for membrane assembly for this protein, was impaired due to small TbTim knockdown. In conclusion, data suggest that the small TbTims are involved in the biogenesis of TbTim17 and other mitochondrial polytopic membrane proteins. Further analyses are ongoing to dissect the role of the small TbTims in mitochondrial protein import.

82 Identification of the Leishmania major U2 snRNA gene promoter

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Leishmania major presents atypical mechanisms of gene expression, such as maturation of mRNAs by trans-splicing. Spliceosomal small nuclear RNAs (snRNAs) are essential for cell viability, since they are required for trans-splicing. Unlike other eukaryotes, in trypanosomatids all snRNAs are synthesized by Pol III. This work is focused on the study of the sequences that regulate transcription of the U2 snRNA in L. major . The U2 snRNA is encoded by a single-copy gene that has a divergently oriented tRNA Ala gene in its 5'-flanking region. The sequence located between these two genes was analyzed allowing the identification of a sequence that resembles a tRNA (tRNA-like), which contains typical A and B boxes. These elements may regulate the expression of U2 snRNA, as observed in T. brucei . Thus, the snRNA gene and 5'-flanking region was cloned into a vector and a tag sequence was inserted in the snRNA gene. Then, progressive deletions and mutations in the 5'-flanking region of the U2 snRNA gene were generated. The expression of the U2 snRNA was analyzed in transiently-transfected cells by qRT-PCR. The results indicated that the A and B boxes of the tRNA-like are indeed required for U2 snRNA transcription. However, unlike T. brucei , the B box from the upstream tRNA Ala gene is also involved in transcription of the L. major U2 snRNA gene. Thus, transcription of the U2 snRNA in L. major seems to require an upstream sequence that is not needed for expression of snRNAs in other trypanosomatids.

83 Co-expression and interaction network analysis of host-trypanosomatid systems

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High-throughput transcriptomics has provided a powerful new approach for studying host-pathogen interactions. While popular techniques such as differential expression and gene set enrichment analysis can yield informative results, they do not always make full use of information available in multi-condition experiments. Co-expression networks provide a novel way of analyzing these datasets which can lead to new discoveries which are not readily detectable using the more popular approaches. We describe the use of co-expression networks to determine important processes and interactions which occur between host and pathogen during the process of intracellular infection. Using time series RNA-Seq datasets for Leishmania major and Trypanosoma cruzi parasites infecting mammalian cells, we describe the construction and annotation of host and pathogen co-expression networks. We also propose a simple approach for detecting statistically significant cross-species co-expression interactions and demonstrate its use in detecting both known and novel interactions between pathogens and their mammalian host cells.

84 Functional analysis of TbMORN1 in bloodstream form T. brucei

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TbMORN1 is a repeat-motif protein that forms a fishhook-shaped macromolecular assembly approximately 2 micrometres long. This protein complex is tightly associated with the trypanosome cytoskeleton, and is situated above the flagellar pocket collar with one end coiled around the flagellum. RNAi-mediated depletion of TbMORN1 in bloodstream form Trypanosoma brucei is rapidly lethal, with a clear difference in the population densities of uninduced and induced cells as early as 12 hours post-induction. Depletion of TbMORN1 results in the appearance of cells with a grossly enlarged flagellar pocket, similar to that seen upon depletion of endocytic mediators such as clathrin, myosin I, and actin. But why should depletion of a cytoskeletal protein that is not on the flagellar pocket membrane cause an apparent defect in endocytic uptake? Fluid phase and lectin reporters provide a clue to the answer.

85 The essential roles of phosphatidylserine synthase 2 and phosphatidylserine decarboxylase in Trypanosoma brucei.

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The membrane phospholipid classes phosphatidylethanolamine (PE) and phosphatidylserine (PS) are ubiquitously present in eukaryotes and prokaryotes. Eukaryotic PE synthesis can occur via i) decarboxylation of PS, ii) head group/base exchange with PS, or iii) the CDP-ethanolamine branch of the Kennedy pathway. Previous work has demonstrated that in Trypanosoma brucei procyclic and bloodstream forms the Kennedy pathway is essential for de novo synthesis of PE. The contributions of the other two pathways for PE production have remained unclear. Similarly, the route for PS formation in T. brucei has not been studied experimentally. We now report the characterization of two (putative) enzymes involved in PE and PS synthesis: T. brucei PS decarboxylase (TbPSD), and T. brucei PS synthase 2 (TbPSS2). Phospholipid analysis in combination with metabolic labeling of parasites after down-regulation or over-expression of TbPSD or TbPSS2 reveal that i) both enzymes are essential for procyclic and bloodstream form normal growth, ii) TbPSD is a mitochondrial type I PS decarboxylase that shows the typcial autocatalytic cleavage of PSD enzymes, iii) TbPSS2 catalyses a reversible base exchange reaction between PE and PS and represents the only route for PS formation in T. brucei. The lack of detectable in vitro or in vivo TbPSD activity suggests that the enzyme may have a different substrate, and function, in T. brucei.

86 High Throughput Phenotypic Screening against Three Kinetoplastid Parasites: An Open Resource of New Compounds Sets

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Three of the Neglected Tropical Diseases (NTDs) declared by the WHO, and included in the L ondon Declaration of 2012, are caused by kinetoplastid parasites. All NTDs have been categorized as “tool ready,” yet also “tool deficient” because many of these tools (i.e. drugs and diagnostics) and implementation strategies are inadequate to achieve the desired goals. New effective, safe, and affordable drugs are needed. We present an integral approach to the early drug discovery for the three major kinetoplastid caused NTDs, i.e. visceral leishmaniasis, Chagas disease and sleeping sickness. The GSK 1.8 million compounds diverse collection has been phenotypically screened against their causative parasites, respectively Leishmania donovani, Trypanosoma cruzi and Trypanosma brucei, using the current state-of-the-art methodologies available in high throughput screening, including genetically modified parasites for screening of intracellular forms of the parasites. Secondary confirmatory and orthogonal assays have been applied in order to confirm anti-parasitic activity and to identify potential cytotoxic activity. Hit compounds have been chemically-clustered and triaged for suitable physicochemical properties. As a result of this effort, three anti-kinetoplastidal boxes of approximately 200 compounds each have been assembled, which represent all the chemical and biological diversity identified and are intended to serve as an open source of starting points for further lead discovery programs, as well as to address important research questions.

87 Molecular characterization of Maf1, putative negative regulator of RNA Polymerase III transcription, in Trypanosoma brucei

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Little is known about transcription regulation in Trypanosoma brucei. One putative transcription regulator is Maf1, a protein identified in Saccharomyses cerevisiae as a repressor of RNA polymerase III (Pol III) transcription in response to multiple stress conditions. In silico analysis of the T. brucei genome revealed the presence of two Maf1 genes (96.2% identical) that contain the three previously-reported Maf1 conserved motifs. RT-PCR assays demonstrated that both Maf1 genes are expressed in T. brucei procyclic forms. To analyze the function of Maf1, RNAi knock-down stable cell lines were generated. After RNAi induction, a 75-90% decrease in Maf1 mRNA level was observed, as well as a 70-85% decrease in Maf1 protein level. Interestingly, induced cultures showed a ~30% increase in cell growth rate, comparing to uninduced cultures. The effect of the Maf1 knock-down on transcription by all RNA polymerases in T. brucei was analyzed by nuclear run-on assays. The results showed a 90% to 180% increase in Pol III transcription. A slight increase in some RNA polymerases I and II transcripts was detected as well. Taken together these data suggest that, similarly to other organisms, Maf1 is a negative regulator of Pol III transcription in T. brucei.

88 Rft1 and protein N-glycosylation in T. brucei

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The membrane protein Rft1 is intimately involved in the synthesis of dolichol-linked oligosaccharides (DLO) needed for protein N-glycosylation. DLO biosynthesis occurs in the endoplasmic reticulum (ER), where dolichol phosphate is sequentially glycosylated to Man5GlcNAc2-PP-dolichol (M5-DLO) on the cytoplasmic side, and then translocated across the ER membrane by a flippase. In the ER lumen, M5-DLO is extended to the mature DLO that is used by oligosaccharyltransferase for protein glycosylation. Yeast genetic analyses identified the ER membrane protein Rft1 as the M5-DLO flippase, but biochemical studies that reconstituted M5-DLO flippase activity in large unilamellar vesicles ruled out this possibility. Thus, the exact function of Rft1 in protein N-glycosylation is still unknown. We previously characterized the growth phenotype and glycosylation profile of T. brucei procyclic forms in the complete absence of Rft1 and showed that despite the nearly-normal growth of the cells, Rft1-null cells accumulate M5-DLO and have decreased levels of Con A-reactive cell surface glycans (1). To elucidate the function of Rft1, we are currently re-introducing tagged and untagged variants of Rft1 into Rft1-null parasites. We assess functionality of the Rft1 variants by their ability to restore N-glycosylation of EP procyclin as well as overall surface glycosylation. Clones expressing tagged functional Rft1 will be used in immunoprecipitation experiments in combination with mass spectrometry analyses to identify proteins that physically associate with Rft1. We anticipate that the identification of these interaction partners will provide clues to Rft1’s function. (1) Jelk, J. et al. (2013) Glycoprotein biosynthesis in a eukaryote lacking the membrane protein Rft1. J. Biol. Chem. 288, 20616-20623.

89 Quantitative Phosphoproteomic Analysis of Stumpy to Procylic differentiation in Trypanosoma brucei

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Trypanosoma brucei lives a complex digenetic lifecycle and its ability to sense and adapt to its host environment is essential for its survival and virulence. The differentiation of growth-arrested stumpy bloodstream form into the proliferating insect-stage procyclic form can be induced synchronously ex vitro by addition of citrate/cis-aconitate (CCA), triggering a phosphatase signalling cascade. Although differentiation proceeds in programmed manner over 72 hours, we show that irreversible commitment to differentiation occurs within 3 hours and is dependent on protein synthesis (see Abstract by Domingo-Sananes et al.). To explore the signalling events underlying commitment, we have utilised a ‘spike-in’ SILAC approach where heavy isotope labelled monomorphic cells as an internal standard for the quantification. Phosphoproteomic and proteomic analysis allowed the quantitative comparison of CCA stimulated and unstimulated stumpy cells after 1h (pre-commitment) and 3h (post-commitment). Significant changes were observed in the abundance of many proteins and phosphorylation sites, including many candidates previously identified as involved in differentiation. A significant number protein kinases and phosphatases displayed rapid changes in phosphorylation upon stimulation, as did potential effector molecules such as RNA binding proteins which typically displayed changes in multiple phosphorylation sites. The changes in phosphorylation engendered by the differentiation stimuli CCA are far wider than the present literature suggests.

90 A Novel Respiratory Protein of Trypanosomatids – A Promising Multi-Parasite Drug Target

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Over 500 million people worldwide are at risk of infection from the trypanosomatids Trypanosoma brucei, T. cruzi and Leishmania spp., and new drugs are urgently required. Although the trypanosome alternative oxidase (TAO) has been widely reported as a drug target in T. brucei, the secondary alternative oxidase (AOX2) has not been investigated. We have recombinantly expressed the AOX2 of all three trypanosomatids in E. coli, and are assaying enzymatic activity and the effects of the traditional TAO inhibitors salicylhydroxamic acid and ascofuranone. Using reverse genetics we have shown that AOX2 is an essential protein in bloodstream form T. b. brucei by creating conditional null mutants, and similar studies are currently underway in T. cruzi and L. major. Furthermore we are identifying the subcellular localization of AOX2 within the parasites and are examining the effects of AOX2 overexpression on cell growth in T. b. brucei. We are comparing the IC50s of wild-type and AOX2-overexpressing T. b. brucei to determine differences in susceptibility to our in-house natural product-like library, to indicate whether AOX2 is the target protein. Finally, we will investigate alternative potential inhibitors of the AOX2 through fragment-based library screening against recombinant AOX2 to identify hits for the AOX2 of all three human-infective trypanosomatids.

91 Do long ncRNAs modulate antigenic variation in Trypanosoma brucei?

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Trypanosoma brucei possesses a tightly regulated mechanism to ensure the mutually exclusive expression of antigens and to switch between different surface antigens, i.e. between different isoforms of variant surface glycoproteins (VSGs). Expression of VSGs can occur from any of ~15 bloodstream-form expression sites (BESs) but only one BES is actively transcribed at a time while all others are transcriptionally repressed. Antigenic variation can be generated by different mechanisms including shifting transcription from one BES to another (in situ switch) and copying a new vsg gene into the actively transcribed BES by duplicative gene conversion. While the trigger leading to in situ switches remains unknown, it was found more than 15 years ago that the sequence upstream of the vsg gene, the co-transposed region (CTR), contains a stabilizing element. Deletion of this region leads to an increase in switching frequency of more than 100-fold. Given the importance of CTRs in ensuring continuous VSG expression, and the role of var associated ncRNA in ensuring monoallelic expression in Plasmodium falciparum , we decided to search for ncRNAs transcribed from BESs. To this end we re-aligned RNA-seq and ribosome-profiling data to the genome of the 427 strain and found the CTR of the active BES to be transcribed into ncRNA. The presence of long ncRNA transcripts was confirmed by northern blotting and RACE experiments. In order to map the location of the stabilizing element more precisely, we plan to systematically delete sub-regions of the CTR and to monitor the effect of segmental CTR deletions on VSG switching.

92 Conservation and function of the 70-bp repeats in Trypanosoma brucei antigenic variation

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Survival of T. brucei in the bloodstream requires that it regularly change its Variant Surface Glycoprotein (VSG) coat to evade the host immune response. To switch coats a silent VSG gene is translocated into an active Expression Site (ES) in a recombination event. Each ES contains a VSG proximal repetitive DNA element termed the 70-bp repeat region, which is thought to function in providing sequence homology during switching. Prior work determined that while these repeats are not required for recombinatorial switching, they are sites of natural DNA breakage, and when an artificial break is placed within the repeats switching is induced. Thus, the actual role of the 70-bp repeats in antigenic variation remained unclear. To better understand the function of these repeats we first analyzed their sequence. Our analysis was consistent with previous reports in terms of length distribution and sequence composition, but uncovered a previously unrecognized level of sequence conservation. We then introduced an I-Sce1 site proximal to the 70-bp repeat region in the active ES, while also genetically manipulating the repeats. Analysis of the outcomes of break-induced switching in the resulting strains showed that 70-bp repeats are required for the selection of VSGs from a variety of sites throughout the genome. We also found that repeat-less strains showed significant delays in cell cycle progression and that, in contrast to previous reports, these strains, upon break induction, were able to switch to very high levels. These experiments suggest that the 70-bp repeats, cell cycle, and switching might be linked functionally and provide a system for the direct exploration of such a link.

93 Transketolase in Leishmania mexicana: Essentiality, localisation and metabolic roles

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The pentose phosphate pathway (PPP) is a major source of NADPH, a reducing agent crucial for defence against oxidative stress as well as other diverse metabolic functions in the cell. Our work is focused on transketolase (TKT), an enzyme of the non-oxidative part of the PPP. We created a TKT knock-out cell line in Leishmania mexicana in order to study its essentiality, localisation and roles in metabolism. In the promastigote stage, the depletion of TKT caused no growth defect, but major changes in metabolism. The PPP is abolished beyond the point of TKT action. Glycolysis is also downregulated and both consumption of glucose and production of metabolic end products decreased, indicating regulatory cross talk between the PPP and glycolysis. Carbon sources other than glucose, however, feed the TCA cycle, most likely a mixture of amino acids. TKT KO cells are of increased sensitivity to oxidative stress, presumably due to decreased NADPH synthesis by the PPP. Interestingly, the deletion of TKT is lethal to the amastigote stage. The cells are non-infective for mice and axenic amastigotes do not survive in culture. Furthermore, we studied the roles and control of the dual, cytosolic and glycosomal, localisation of TKT. Constructs that targeted the enzyme either exclusively to the glycosomes or to the cytosol had no effect on viability, oxidative stress sensitivity nor metabolism of the cells. In addition to the fine details on the TKT action, the work implies interesting mechanisms about control and adaptation of leishmania metabolism in general.

94 Target identification of anti-trypanosome compounds using an over-expression library

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Knowledge of the mechanism of action of compounds against Trypanosoma brucei is crucial for medicinal chemistry and studies regarding side effects and resistance. High-throughput screens are revealing new chemical molecules with anti-kinetoplastid activity and unknown target. Current methods for target discovery are not always effective, therefore new complementary methods are needed. A large inducible overexpression library was established in T.brucei, containing random genomic DNA fragments. The library is large enough to ensure several-fold in-frame coverage of protein coding regions. The transfected parasites were used in cultures containing drugs and the surviving trypanosomes were screened for candidate genes conferring growth advantage. The overexpressed library inserts could encode direct targets or genes involved in the drug metabolism. As proof of principle, the library was selected with difluoromethylornithine and DDD85646; their respective targets were identified, ornithine decarboxylase and myristoyl-CoA-protein N-myristoyltransferase. New promising compounds were tested. Although few candidate genes were found, the full length gene overexpression did not confer growth advantage. In these cases other factors probably caused resistance, for example mutations in the genome. Therefore other techniques will be used for further studies. The approach was shown to work with two compounds, as proof of concept. Even though our library did not reveal new targets so far, an improved version overcoming present issues could be a powerful tool for target discovery and other applications.

95 Cell differentiation in Trypanosoma brucei is a bistable developmental switch requiring new protein synthesis to achieve signal memory

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The trypanosome life-cycle involves several developmental transitions that allow transmission, survival and proliferation of these parasites. One of these, the differentiation of growth-arrested stumpy forms in the mammalian blood into proliferating insect-stage procyclic forms, can be induced synchronously in vitro by addition of cis-aconitate (CA). Using single-cell analysis by flow-cytometry we formally show that this transition is an irreversible bistable switch where cells commit to differentiation after 1-3 hours of exposure to CA. This irreversibility implies the existence of positive feedback mechanisms that allow commitment: i.e. the establishment of "memory" of exposure to the differentiation signal. Such mechanisms probably depend on post-translational modifications and/or the synthesis of regulatory proteins. Using the reversible protein synthesis inhibitor cycloheximide, we show that protein synthesis is required for establishment of signal memory and normal commitment to differentiation. To determine the underlying protein synthesis and signalling events in parasites undergoing synchronous differentiation we performed SILAC phosphoproteomics, this providing a detailed map of the ‘commitment phosphoproteome’ (refer to the accompanying abstract cited below). Finally, we employed a rigorous candidate gene approach to demonstrate that the stumpy-enriched protein kinase TbNRKA/B stringently controls the initiation of differentiation to procyclic forms, identifying this kinase as a major regulator of the earliest events in trypanosome development. Combined these studies provide a detailed cytological, molecular and regulatory framework for trypanosome differentiation likely to be relevant for the developmental events intrinsic to the life cycle events of many protozoan parasites. Accompanying abstract: Quantitative Phosphoproteomic Analysis of Stumpy to Procylic differentiation in Trypanosoma brucei Michael D. Urbaniak, Maria Rosa Domingo-Sananes, Balazs Szoor, M. A. J. Ferguson and K. R. Matthews.

96 A toolkit enabling efficient, scalable and reproducible gene tagging in trypanosomatids

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The development of high throughput methods to analyse the DNA, RNA and protein content of cells has created large datasets that contain numerous proteins of interest. A common first step in analysing protein function is to determine protein localisation using a fluorescent tag; however, the methods for tagging proteins have not kept pace with the developments in genomics, transcriptomics and proteomics, creating a bottleneck in the analysis of these datasets. We have developed rapid, reproducible, high-throughput, PCR-based tools for endogenous tagging of genes in Trypanosoma brucei (Dean S, Sunter J , Wheeler RJ, Hodkinson I, Gluenz E, Gull K. (2015) A toolkit enabling efficient, scalable and reproducible gene tagging in trypanosomatids. Open Biol. 5: 140197.). We generated a single plasmid that when coupled with long primer PCR can be used for both N- and C-terminal tagging of genes at the endogenous locus in procyclic and bloodstream form Trypanosoma brucei . Furthermore, protein truncation experiments and gene knockouts can be undertaken using the same PCR approach. Long primer PCR is not effective in Leishmania mexicana . Hence, we developed a fusion PCR approach suitable for use in trypanosomatid species with less efficient homologous recombination. We have optimised the workflow and protocols making it efficient, scalable and reproducible, enabling the tagging of genes at scale on 96-well plates. Hence, the tagging of every protein-coding gene in the T. brucei genome is now a readily achievable goal. We have produced a range of plasmids with different combinations of tags (fluorescent and epitope) and resistance genes. We will be distributing the plasmids for endogenous gene tagging in T. brucei and Leishmania spp here at the conference.

97 TARGET-BASED DISCOVERY OF NOVEL PAULLONES WITH ANTI-LEISHMANIAL ACTIVITY

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Leishmaniasis is a devastating disease for which the current treatment is far from optimal due to toxicity, limited efficacy and development of resistance. Thus, the identification of novel drug-like compounds targeting essential and unique components of the parasite metabolism is a priority. In this respect, trypanothione synthetase (TryS) appears as a suitable drug-target because it is exclusive of Kinetoplastida and produces trypanothione (bis-glutathionyl spermidine), a low molecular weight thiol that is indispensable for pathogenic trypanosomatids. Whilst seeking for TryS inhibitors, we have identified N5-substituted paullones (benzo[2,3]azepino[4,5- b]indol-6-ones) as potent (nM) and selective inhibitors of TryS from Leishmania infantum . The biological activity towards L. braziliensis , the major causative agent of human cutaneous leishmaniasis in Central and South America, and murine macrophages (cell line J774) was evaluated for the most active anti-TryS compounds (IC 50 = 1 μM). This led to the identification of KuRK259 and KuRK278 as potent (EC 50 low or sub-μM) and selective (selectivity index > 50) growth inhibitors of intracellular amastigotes. Strikingly, the screening against infective (late stationary phase) promastigotes revealed other related paullones (KuRK 130 and KuRK 269) as anti-proliferative agents, albeit, at concentrations 10-fold higher (e.g. EC 50 ~10 μM). The lack of correlation for the activity of the compounds against promastigote and amastigote cells is in agreement with the different metabolic needs of each life- and growth-stage. In summary, we have identified novel drug candidates displaying a potent and selective inhibition of leishmanial TryS and of L. braziliensis amastigotes growth. Future work will address the therapeutic efficacy of the most promising paullones.

98 Post- translational regulation of Leishmania aquaglyceroporin AQP1

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Ubiquitin-proteasome mediated and lysosomal proteolysis are two major pathways for intracellular protein degradation. Ubiquitinated proteins are destined to be degraded by proteasomes. Leishmania has an E3 ubiquitin ligase called Anaphase Promoting complex/Cyclosome (APC/C) homologue in its genome. APC/C recognizes its substrates by RXXL motifs. We found two RXXL motifs at the free cytosolic N-terminus of AQP1 and a putative ubiquitination site specific lysine (K12). Levels of aquaglyceroporin AQP1 expression determine the osmoregulation capacity and antimony sensitivity profile of Leishmania. We reported that Mitogen activated protein kinase 2 (MPK2) regulates AQP1 stability post-translationally through phosphorylation at threonine 197. We found that lysine 42 (K42) is critical for MPK2 and, cells co-overexpressing AQP1K42AMPK2 showed slower osmoregulation compared to cells co-overexpressing wild type proteins. Our aim was to identify whether K12 is the actual site of ubiquitination and its effect on the stability of AQP1 in the presence or absence of MPK2. During osmoregulation, we observed that cells overexpressing single and double mutants for R 20 XXL 23 and R 27 XXL 30 motifs were less efficient osmoregulatory compared to cells overexpressing wild-type AQP1, suggesting lower expression of altered proteins compared to wild type. Whereas, cells co-overexpressing K12AAQP1 and K12RAQP1 with MPK2 and K42AMPK2 showed increased osmoregulation capacity compared to AQP1K42AMPK2 suggesting higher expression of AQP1 which corroborated with antimony sensitivity profiles of the transfectants. Taken together, our data suggested that (i) in the absence of phosphorylation, ubiquitination at K12 is required for the degradation of Leishmania AQP1 and, (ii) RXXL motifs are not involve for ubiquitination at K12.

99 Cargo selection in the early secretory pathway of Trypanosoma brucei

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In African trypanosomes, trafficking of GPI-anchored Variant Surface Glycoproteins (VSG) to the cell surface, and digestive hydrolases to the lysosome, are critical to parasite survival and pathogenesis. In the first step of trafficking, nascent secretory proteins in the ER are selected into COPII-coated vesicles departing from ER Exit Sites (ERES). In other eukaryotes, the p24 family of transmembrane proteins form heteromeric complexes that interact simultaneously with COPII components (Sec23/24 heterodimer) on the cytoplasmic side, and secretory cargos (soluble & GPI-anchored) on the lumenal side, of budding vesicles. In trypanosomes, ER exit of VSG is mediated by a subset of COPII components (TbSec23.2/TbSec24.1), suggesting a VSG-sorting receptor during COPII vesicle formation. We hypothesize that VSG (and/or other cargos) are selected for ER exit by trypanosomal p24 orthologues. Querying the T. brucei genome with yeast EMP24 identified 8 putative p24 genes (TbERP1-8, EMP24-Related Protein). Thus far, we have evaluated TbERP1,2,3,8 by RNAi silencing. Of these, TbERP2 silencing caused a moderate growth defect. However, silencing of TbERP1 delayed the trafficking of VSG to the cell surface (~2-fold). Additionally, silencing of TbERP1,2,3 or 8 delayed (2-4 fold reduction) trafficking of TbCatL (soluble hydrolase) to the lysosome. Immunolocalization confirms that TbErp1,2,3 & 8 localize to ERES. These TbErps show inter-dependent protein stability; each TbErp1,2,3 or 8 is destabilized by specific RNAi silencing of the other TbERP genes. The overlapping RNAi phenotypes, localization, and coordinate expression of these TbErps suggest complex(es) with novel specificity(s), and experiments are underway to confirm direct physical interactions. Work is also ongoing to evaluate the role(s) of the uncharacterized TbERP4-7 in the trafficking of VSG.

100 A glycosylation mutant of Trypanosoma brucei links social motility defects in vitro to impaired colonisation of tsetse in vivo

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Transmission of African trypanosomes by tsetse flies requires that the parasites migrate out of the midgut lumen and colonise the ectoperitrophic space. Early procyclic culture forms correspond to trypanosomes in the lumen; on agarose plates they exhibit social motility, migrating en masse as radial projections from an inoculation site. We show that an Rft1-/- mutant needs to reach a greater threshold number before migration begins, and that it forms fewer projections than its wild-type parent. The mutant is also up to 4 times less efficient at establishing midgut infections. Ectopic expression of Rft1 rescues social motility defects and restores the ability to colonise the fly. These results are consistent with social motility reflecting movement to the ectoperitrophic space, implicate N-glycans in the signalling cascades for migration in vivo and in vitro, and provide the first evidence that parasite-parasite interactions determine the success of transmission by the insect host.

101 Functional characterization of amino acid transporters in T. brucei

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In trypanosomatids amino acids are required for various processes such as synthesis of proteins, energy production, cell cycle transition, polyamine synthesis and osmoregulation. Several amino acids cannot be synthesized by the parasites and therefore have to be imported, making amino acid transporters (AATs) interesting drug targets or drug delivery systems. In the T. brucei genome multiple AAT genes have been identified [1]. To functionally characterize T. brucei AATs, the ORFs were expressed in Saccharomyces cerevisiae mutants and growth on amino acids was evaluated. This approach revealed potential substrates for many AATs. Heterologous expression of members of the AAT5 and AAT16 loci in S. cerevisiae promoted growth on medium supplemented with arginine, lysine or histidine as sole source of nitrogen or histidine, respectively. Moreover, transport studies showed that AAT5 members were selective for arginine and mediated high-affinity arginine uptake, whereas gene products of the AAT16 locus mediated high-affinity transport of lysine. Down-regulation of AAT5 by RNAi in bloodstream form trypanosomes indicated that the carrier is essential for growth in culture. The growth arrest could be reversed by excess arginine, but not ornithine or citrulline. Similarly, RNAi against AAT16 produced a strong growth defect in bloodstream form T. brucei, which was prevented by supplementing lysine in the growth medium. Interestingly, Northern blot analyses of AAT5 RNAi lines demonstrated an increased level of AAT16 mRNA and vice-versa, i.e. down-regulation of AAT16 promoted up-regulation of AAT5 transcript levels. Our results provide a detailed characterization of members of two AAT loci and show that T. brucei bloodstream forms regulate AAT mRNA levels. [1] Jackson, AP (2007) BMC Evolutionary Biology 7.

102 Ectopic VSG overexpression: A tool to identify new players in the regulatory network of the expression site.

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The bloodstream form (BSF) of Trypanosoma brucei thrives extra-cellularly in the blood of its mammalian host where it is present as two distinct life cycle stages: the proliferative long slender (LS) and the cell cycle arrested short stumpy (ST) form, which is infectious for the tsetse fly (1) . At any given time, the BSF expresses only one out of hundreds of variant surface glycoproteins (VSGs) in a monoallelic manner from one of about 15 telomeric expression sites (ES) (2) . Recently, we revealed a link between monoallelic VSG expression, ES activity and the development from slender to the transmissible ST form (3) . We showed that ectopic expression of a second VSG causes a gradual attenuation of the active ES and concomitantly a transient gain of developmental competence. However, the commonly used laboratory strains have lost the capability to naturall y differentiate. Therefore, we established the inducible ES attenuation tool in a trypanosome strain (AnTat1.1) that still possesses full differentiation potential in order to further investigate the role of ES activity in the LS to ST stage transition. We show that VSG overexpression leads to the development of bona fide stumpy cells that possess the capacity to progress in the parasite cycle. Further, we could demonstrate that the ectopic overexpression of two different VSG proteins induces stumpy development, underlining the biological relevance of our model. We are now using the ES attenuation tool in combination with state of the art high throughput methods to identify new players in the ES regulatory network. 1) Rico, E., 2013, Frontiers in Cellular and Infection Microbiology, 3: 1-15 2) Günzel, A., 2014, 556(1):68-73 3) Batram, C., 2014, eLife, 3: e02324

103 Characterisation of the interaction between the haptoglobin-haemoglobin receptor and its ligands in the context of the trypanosome cell surface.

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The haptoglobin-haemoglobin receptor (HpHbR) of African trypanosomes mediates uptake of haem from the host through binding and internalisation of haptoglobin-haemoglobin (HpHb). The receptor also plays a central role in human innate immunity to trypanosome infection as it is the route of uptake for trypanolytic factor-1. The HpHbR is a GPI-anchored surface protein, present in low copy number, which must function for ligand uptake in the context of the dense VSG surface coat. The structure of the Trypanosoma brucei HpHbR bound to human HpHb reveals a kink in the receptor that exposes the ligand binding sites and allows dimeric binding of two receptors to one HpHb dimer. Comparison of ligand uptake into cells reveals that receptor-mediated uptake of dimeric HpHb occurs at lower concentrations than monomeric HpHb thus confirming increased ligand avidity though bivalent binding in live cells. The resulting high affinity for binding and uptake may be required to enable trypanosomes to compete for HpHb with the CD163 scavenger receptor on host macrophages.

104 Ultrastructural defects in Leishmania mexicana KHARON1 null mutant amastigotes

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KHARON1 (KH1) is a kinetoplastid-specific protein involved in the trafficking of the glucose transporter LmxGT1 to the flagellar membrane in Leishmania mexicana promastigotes. The short flagellum present in the amastigote stage differs structurally from that of the promastigote; it lacks structures necessary for motility and resembles sensory cilia but its function remains unclear. Surprisingly, KHARON1 was shown to be critical for the viability of L. mexicana amastigotes within macrophages, but its precise role in this life cycle stage is currently unknown. To address the cause of the reduced viability in the intracellular amastigote stage and investigate whether the structure of the flagellum was affected, transmission electron microscopy was used to compare ultrastructural phenotypes of KH1 null mutant promastigotes, axenic amastigotes and intracellular amastigotes with the wild type. This analysis revealed amastigote-specific ultrastructural phenotypes in the form of distended flagellar tip structures. KH1 null mutant intracellular amastigotes additionally showed a rounded cell shape as compared to wild type cells, and we observed examples of cells with supernumerary nuclei, but no evidence of cleavage furrow formation or cell-shape remodeling. This suggests an inability to undergo cytokinesis. The causal relationship between the different ultrastructural phenotypes and the failure of cytokinesis remains unclear, but elucidation of this may provide a mechanistic explanation for the inability of the parasites to survive within the host macrophage as well as insight into the role of the amastigote flagellum.

105 Investigating the complexity and dynamics of the minicircle repertoire in *Trypanosoma brucei*

***Schnaufer, Achim (University of Edinburgh)**; Cooper, Sinclair (University of Edinburgh); Savill, Nicholas (University of Edinburgh)*

Uridine insertion/deletion editing of mitochondrial mRNAs is essential in Trypanosoma brucei . Minicircle DNA molecules encode the vast majority of the numerous different guide RNAs (gRNAs) that contain the sequence information for RNA editing, while mRNAs are encoded by the maxicircle component of the trypanosome mitochondrial DNA. Minicircles and maxicircles are interlinked in a massive DNA network, the kinetoplast (or kDNA). T. brucei minicircles are diverse, partially redundant and of variable copy number. As a result of imprecision in the minicircle replication and segregation mechanism, copy numbers are dynamic. The precise extent of diversity and redundancy, and how quickly this can change over time, is unknown. The life cycle stages of T. brucei in the mammal and the tsetse depend on different subsets of mitochondrial mRNAs, and therefore the parasite faces the challenge of maintaining full editing capacity in the absence of immediate selection. We have now elucidated the full complexity of kDNA in T. brucei AnTat 1.1 through deep sequencing. We identified gRNA genes for the entire known editing space, show that there are more sequence classes than previously thought, and discovered a nucleotide bias for these sequences that allowed us to predict non-canonical gRNA genes. In a pilot study we found loss of several minicircle classes over a surprisingly short time frame. This finding suggests that redundancy in the gRNA repertoire serves an important buffering function to maintain the parasite’s capacity for life cycle progression. We are now carrying out a larger study that, with the help of a mathematical model, will accurately determine the precision of minicircle replication/segregation, an important aspect of the transmission dynamics of T. brucei .

106 The unconventional nuclear envelope of kinetoplastid flagellates

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The nuclear envelope (NE) is a defining structure of the eukaryotic cell. Prominent NE structures are nuclear pore complexes (NPC) and the filamentous lamina underlying the NE. In addition to structural functions, the lamina interacts with an extensive cohort of proteins, which in metazoan cells include LEM domain proteins, that together with BAF and lamin B receptor operate in chromatin tethering and LINC complexes that serve as bridges that connect the nucleoskeleton to the cytoskeleton. Until recently these structures were only known from model organisms such as mammalian cells and yeast that belong to a relatively narrow eukaryotic group Opisthokonta. Here we provide more insight into the evolution of the NE from pan-eukaryotic homology searches, phylogenetic analyses of the individual NE components and experimental data on the composition of the NE from the diverged eukaryotic parasite Trypanosoma brucei. Although the overall structure and composition of the NPC is similar between trypanosomes and other eukaryotes, several subunits are kinetoplastid-specific. The multiple functions of trypanosome nuclear basket overlap with the basket proteins of opisthokonts despite distinct origins. Similarly, the trypanosome lamina is composed of proteins restricted to kinetoplastids but the two major protein components, NUP-1 and NUP-2 have functions analogous to lamins. Moreover, NUP-1 is similarly to lamins phosphorylated by a CDK1-related kinase, which is important for faithful separation of daughter nuclei during mitosis. The divergence of kinetoplastid flagellates is further underlined by the apparent absence of LINC complexes and other integral NE proteins, which are otherwise conserved among major eukaryotic lineages.

107 *Trypanosoma brucei* RNA editing ligase 1 (REL1) is involved in the religation of deletion, insertion, and non-canonical editing sites *in vivo*

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Uridylyl (U) insertion/deletion RNA editing in Trypanosoma brucei is governed by multiprotein complexes called editosomes. The final step in each round of editing is religation of mRNA fragments. The ~20S core editosomes (or RNA editing core complexes, RECC) contain two ligases, REL1 and REL2, associated with the U removal enzyme REX2 and the U addition enzyme RET2, respectively. While REL1 is clearly essential for RNA editing, REL2 knockdown by RNAi has not resulted in a detectable phenotype. To explain these findings, alternative scenarios have been suggested: (a) REL2 is not functional in vivo; (b) REL1 can function in both insertion and deletion editing, whereas REL2 can only function in insertion editing; (c) REL1 has an additional role in repairing mRNAs cleaved at incorrect sites. We have now investigated the function of REL1 by quantitative analysis of mRNA fragments that accumulate in vivo after ablation of the enzyme. This analysis was enabled by developing a novel deep sequencing protocol and a bioinformatics pipeline for mapping the highly complex editing intermediates. Our results demonstrate that REL1 is important for religation of mRNAs at both deletion and insertion editing sites. Repression of REL1 also results in accumulation of mRNAs cleaved at non-canonical sites, suggesting that the enzyme has an additional role in religating mRNAs cleaved at the wrong sites. Evolutionary analysis of the REL2 sequence shows that the enzyme is under strong purifying selection, despite lack of experimental evidence for an important function. We propose that REL2 serves a proofreading role in RNA editing, while REL1 is the ‘work horse’ that is responsible for re-ligation of most canonical and non-canonical editing events in vivo .

108 Integrating a new screening strategy and insights from crystallography for drug discovery against Trypanosoma brucei.

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Sleeping sickness has a global health burden similar to multiple sclerosis, but a comparatively tiny drug pipeline (2 clinical candidates compared to 60 for MS). Phosphodiesterases B1 and B2 are essential proteins in the bloodstream form of Trypanosoma brucei that appear tractable for drug discovery. However, the development of drug leads has been hindered by problems achieving potency with selectivity for TbrPDEBs versus human PDEs. To overcome these problems we have implemented two related lines of research. The first is to discover new chemotypes with high potency against T. brucei bloodstream strain Lister 427, selectivity versus human cells, and selective potency against TbrPDEB1. We screened 30,000 hits from a wild-type T. brucei whole cell screen against the PDE inhibitor-resistant T. brucei strain R0.8, and counter-screened the most promising compounds against human HepG2 cells. This enabled us to prioritize selective compounds with sub-micromolar potency against T. brucei and evidence suggesting a mechanism of action related to cAMP signaling. These compounds are being tested for inhibition of TbrPDEB1. The second line of research is directed at understanding structure-activity features of the T. brucei PDEB1 active site that can be exploited to improve compound selectivity. We have solved the structure of T. brucei PDEB1 bound to the repurposing hit piclamilast. We have used this structure and chimeras of human PDE4 and T. brucei PDEB1 to identify binding site features that influence ligand binding. This synergistic approach of screening for new inhibitor chemotypes as well as understanding structure-activity relationships of hit series will augment efforts to effectively target the PDEBs in T. brucei , and add new drug candidates to the pipeline for sleeping sickness.

109 Evolution of membrane trafficking in kinetoplastids

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Life-cycle progression and pathogenic mechanisms in kinetoplastid parasites depend on their intracellular trafficking systems, to facilitate rapid remodelling of surfaces to evade the host immune system. Genome-wide sequence data for many parasitic kinetoplastids are becoming available, along with the free-living Bodo saltans. We have used these resources to reconstruct how intracellular trafficking varies across this lineage, selecting three central protein trafficking families SNAREs, Rab GTPases and TBC-domain containing proteins. In parallel, we used cryoimmunoisolation, proteomics and imaging in Trypanosoma brucei (Tb) to study members of the SNARE protein family. We find that overall SNARE, Rab GTPase and TBC protein families are stable across the kinetoplastids, with little evidence for large-scale expansions or losses accompanying either parasitism, alterations in host environment and defence systems or transmission mechanisms. However, many significant smaller scale changes are evident, suggesting that small scale modulation of trafficking systems has accompanied the evolution of these species. Proteomics and tagging demonstrates that TbSNAREs orthologous to SNAREs in yeast and metazoan have similar subcellular localisations in Trypanosoma brucei . We find that specific intra-SNARE interactions that mediate their function at each stage of the trafficking pathway are also largely conserved in the complexes we have studied. Further, we find that the interactions of TbSNAREs with SM (Sec1/Munc18-like) proteins, their main regulators are also conserved. Overall, while there is evidence for Rab protein evolution enabling modification of trafficking pathways, the SNARE protein family are remarkably stable.

110 The mRNA-Bound Proteome in Trypanosoma brucei: towards a comprehensive regulatory network

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RNA-binding proteins (RBPs) play an important role in controlling the life of mRNAs. Until recently, annotation of RBPs was limited to proteins with known RNA-binding domains (RBDs) and their effects on mRNA function were unknown. We have set out to provide a comprehensive overview not only of the proteins bound to mRNAs, but also their functions. In a Trypanosoma brucei whole-genome "tethering" screen, in which random protein fragments were artificially bound to a reporter mRNA, we previously identified over 300 proteins potentially implicated in post-transcriptional mRNA regulation. We have now created a small-scale ORFeome containing the most promising potential regulators. Employing the tethering approach again, we have confirmed reproducible enhancement or suppression of gene expression by more than 100 proteins. These included canonical RBPs and also novel potential regulatory factors. Meanwhile, a corresponding proteome array identified unexpected RNA-binding activities. To find out which proteins are bound to mRNAs in vivo, we captured poly(A)+-mRNA-bound proteins from bloodstream forms. The mRNA-bound proteome included about 80 proteins including 33 RBDs-containing proteins. Interestingly, these included not only proteins that increase gene expression in the tethering assay, such as PUF9, ZC3H11, and RBP42, but also some that suppress it, such as UPF1, RBP10, and PUF2. However, there are also about 20 proteins with unknown functions. Notably, seven of these had previously been identified as regulators in the tethering screen and four have been found in our protein array as RNA binders. Identification of the mRNA targets of the regulatory mRNA-bound proteins, and their interactions with the degradation machinery, is now in progress and should increase our understanding of the gene expression regulatory network in trypanosomes.

111 The nuclear protein UMSBP interacts with its mitochondrial paralogue and is involved in kinetoplast DNA replication and telomeric binding.

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Kinetoplast DNA (kDNA) is the mitochondrial genome of trypanosomatids. Its major constituents are several thousand duplex DNA minicircles whose replication origins are bound by the CCHC-type zinc finger protein UMSBP. Simultaneous knockdown of the two UMSBP orthologue genes of Trypanosoma brucei (TbUMSBP1 and TbUMSBP2) inhibited the initiation of kDNA replication and segregation. Immunofluorescence analysis, using genomically tagged proteins, revealed that while TbUMSBP1 is localized to the mitochondrion, TbUMSBP2 has a nuclear localization. In accord with their distinct localization and functions, binding kinetics analyses revealed that the mitochondrial TbUMSBP1 displays a significantly higher affinity than the nuclear TbUMSBP2 to the conserved kDNA origin sequences, as well as lower capacity to remodel kDNA networks, condensed by the kinetoplast associated proteins (KAPs). Moreover, depletion of TbUMSBP2 resulted in impaired nuclear division and accumulation of DNA in the nucleus, leading to cell growth arrest. Fluorescence in situ hybridization analyses combined with immunofluorescence revealed the co-localization of TbUMSBP2 with chromosomal telomeres. Knockdown of TbUMSBP2 results in the loss of telomeres nuclear peripheral localization and compromises the ability of telomeres to repress DNA damage response. These results indicate that TbUMSBP2 play an essential role in telomere maintenance and function in trypanosomes.

112 Regulation of kDNA Replication Initiation by Post Translation Modifications

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The universal minicircle sequence binding protein (UMSBP) is a DNA binding protein which functions in kDNA replication and segregation. UMSBP activity is affected in vitro by the trypanothione-dependent redox regulating enzymes, tryparedoxin (TXN) and tryparedoxin peroxidase (TXNPx). TXN reduces UMSBP, activating its origin-binding capacity, whereas TXNPx oxidizes UMSBP, impairing this activity. Two genes, encoding known mitochondrial trypanothione-dependent peroxidases: TRYP2 (which encodes mitochondrial TXNPx) and glutathione peroxidase-like 3 (GPX3) , were down regulated using either inducible RNAi or gene knock out strategies. GPX3 knockout cells show an increased level of minicircles replication while TRYP2 knock down cells show no significant change in minicircle replication. 13% (15/116) of the amino acids in UMSBP are cysteines. To determine the redox reactive cysteine residues, we used thiol trapping and mass spectrometry analyses. UMSBP is phosphorylated in vivo in a cell cycle-dependent manner. To study the role of phosphorylation in UMSBP regulation, we enriched phosphorylated UMSBP from C. fasciculata using titanium dioxide affinity chromatography. Tandem MS analysis identified serine 83 as the phosphorylated amino acid. Site-directed mutagenesis, substituting serine 83 in UMSBP by glutamate (a phosphomimetic amino acid) or by alanine, revealed that both mutants exhibited reduced DNA binding activities, in comparison to that of the wild type protein. This result suggested that phosphorylation of UMSBP has an inhibitory effect. This conclusion was further challenged by comparing DNA binding activity of endogenously phosphorylated UMSBP with unphosphorylated protein, revealing that the activity of endogenously phosphorylated UMSBP was lower than that of an unphosphorylated protein.These results shed light on the role of post-translation modifications in the regulation of UMSBP activity in trypanosomes.

113 Is Vitamin C Biosynthesis essential in Trypanosoma cruzi?

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Vitamin C (ascorbate) is a redox-active carbohydrate that plays a critical role in many eukaryotic metabolic pathways. In humans, it is required for collagen and neurotransmitter biosynthesis. In addition, it is involved in antioxidant defence. It can metabolise reactive oxygen species, donate electrons to ascorbate-dependent peroxidases and participates in iron-uptake, which, in trypanosomes, is required for superoxide dismutase activity. Most eukaryotes synthesize ascorbate from sugar precursors, L-gulonolactone in mammals and L-galactonolactone in plants. Trypanosomes cannot take up ascorbate from the environment and express a glycosomal enzyme capable of converting either L-galactonolactone or D-arabinonolactone to ascorbate or erythroascorbate, respectively. Neither enzyme can use L-gulonolactone as a substrate, differentiating them from the mammalian equivalent. In T. brucei the genes for this enzyme (TbALO) were readily deleted and null mutants showed no significant phenotype. We therefore examined whether T. cruzi could also survive without an ascorbate biosynthetic capacity. Attempts to delete both copies of TcGAL were only successful using parasites where TcGAL activity was supplied by an episomal copy. However, when these cells were then removed from drug selection for 90 generations, we were able to isolate null mutants that had lost the episomal copies of TcGAL. Clonal lines were derived from these populations for further characterization. Their phenotype will be reported including infectivity as assessed by highly sensitive bioluminescent imaging in an experimental model of Chagas disease.

114 ARM58, AN ANTIMONY RESISTANCE MARKER IN LEISHMANIA SPP

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ARM58 was identified as a novel marker for antimony resistance [1]. Over expression protects leishmaniae against antimony during all stages by lowering the intracellular antimony concentration. ARM58 comprises four conserved domains of unknown function (DUFs). A deletion analysis identified DUF-3 as essential for drug resistance [2]. The protein protects against antimonials, but not against arsenite, pentamidine or miltefosine [2]. In over expressing strains, ARM58 shows 5-fold increased above wild type levels and can be detected using a specific anti-ARM58 IgY. Under denaturing conditions, the antibody detects a major protein band corresponding to a molecular mass of ~75 kDa, but under native conditions the protein migrates as 58 kDa species. In spite of its predicted TMD, ARM58 is a soluble protein that is recovered mainly with the soluble lysate. ARM58 over expression is detected in the secreted fraction, suggesting that the protein is secreted from the cell. Trypsin and TritonX100 treatment of this fraction shows ARM58 excreted in vesicles. Indirect immune fluorescence microscopy using anti-ARM58 IgY reveals an almost exclusive flagellar staining both in wild type L. infantum and upon ARM58 over expression. FACS analysis with SYTOX shows that ARM58 over expression protects cells against antimony-induced DNA fragmentation. 1. Nühs, A., et al ., Int. J. Parasitol: Drugs and Drug Resistance, 2014. 4: p. 37-47. 2. Schäfer, C., et al ., Antimicrob Agents Chemother, 2014. 58: p. 1565–1574.

115 Ordering components of the slender to stumpy signalling pathway in Trypanosoma brucei

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In the mammalian bloodstream, Trypanosoma brucei undergo differentiation from proliferative slender forms to arrested, transmissible, stumpy forms. This transition is associated with extensive cytological and metabolic changes that promote survival in the tsetse midgut, and also influences infection dynamics within the mammalian host. A number of genes involved in this transformation were recently identified using an RNAi library screen for resistance to pCPTcAMP, a membrane-permeable cyclic AMP analogue that induces differentiation. These molecules were thereafter validated to regulate the slender to stumpy transition in vivo, with many of them apparently comprising part of a signal tranduction and effector pathway (1). However, it is unknown how these proteins act in relation to one another or are ordered in the pathway. To this end, we have created null mutants for several of the identified genes in pleormorphic trypanosomes and, in this genetic background, expressed other members of the predicted pathway to test their ability to restore stumpy formation. This extragenic suppression approach has also been applied to explore the interaction between the identified drivers of stumpy formation and the unusual kinetoplastid-specific target of rapamycin kinase, TOR4, which has previously been shown to act as a negative regulator of stumpy formation in monomorphs. Finally, RNAi lines for the different components of the stumpy formation pathway have been tested for their ability to undergo development in the presence of the recently identified differentiation-inducing compound DDD00015314 (2). This combined chemical-genetic approach is building a pathway structure for the signalling events underlying trypanosome quorum sensing. 1. B. M. Mony*; P. MacGregor*; et al. (2013) Nature doi:10.1038/nature12864 2. P. MacGregor et al. (2014) Eukaryotic Cell, 13, 412-426

116 TAC102 a novel component of the Tripartite Attachment Complex (TAC)

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Identification and characterization of the mitochondrial DNA segregation machinery has been a long-standing question. We present a novel component of the Tripartite Attachment Complex (TAC). TAC102 is a ~100kDa structural protein targeted to the unilateral filaments of the TAC. Targeting of TAC102 to the mitochondrion does not depend on a N-terminal targeting sequence, rather the C-terminus of TAC102 seems to be key for proper localization. Loss of this novel TAC component leads to unequal segregation of the kDNA with the new basal body being unable to properly connect to the kDNA, while the old basal body remains connected. As a consequence the majority of cells become diskinetoplastic and a few cells accumulate large amounts of kDNA. Ultrastructure analysis demonstrate that the kDNA in these cells is by and large properly organized however multiple kDNA discs are now crowded in one kDNA pocket. Loss of TAC102 does not impair proper mitochondrial organelle segregation suggesting that the two processes are not connected. Furthermore loss of TAC102 also does not directly impair replication of mini- or maxicircles.

117 The kinetics of folate and antifolate drug transport in Trypanosoma brucei

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Trypanosomatids lack a de novo folate-synthesis pathway and are dependent on exogenous folate for growth. Folate transport has been studied in Leishmania species; but little is known regarding folate transport in Trypanosoma brucei. In-vitro studies have shown that folate levels in medium modulate the sensitivities of certain antifolate drugs. The reason why high folate levels were found to reduce the sensitivity of antifolates is yet to be determine but hypotheses include folate competitively inhibiting drug uptake into cells, or competing for the active sites of target enzymes. We went on to study folate uptake in T. brucei. A rapid transport assay was developed where bloodstream trypanosomes are mixed with radiolabelled folic acid or methotrexate and centrifuged through dibutyl phthalate at different time intervals. Uptake is time, concentration and temperature-dependent with linear rates of uptake over the first 150 seconds. Transport of both folate and methotrexate obey simple Michaelis-Menten kinetics. Folate transport was inhibited by methotrexate, pemetrexed and raltitrexed (classical anti-folates that are folate structural analogues) and 5-methyl THF (the principle form of folate in human plasma). Conversely methotrexate transport was inhibited by folate, 5-methyl THF and classical antifolates. Lipophilic (non-classical) antifolates had no effect. More detailed kinetic studies revealed that methotrexate inhibits transport of folate by acting as a competitive substrate, rather than a competitive inhibitor. Our results indicate that the classical anti-folates, 5-methyl THF and folate likely share a common transporter (s) and that the modulation of cell potency of classical antifolatess by folate can be (largely) explained by competition for uptake into the parasite. Using inducible RNAi we are generating knock-down mutants to identify the putative folate transporter genes involved.

118 Ion channels in Trypanosoma brucei as potential drug targets

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Ion channels in the plasma membrane can be involved in essential regulatory mechanisms and are easily accessible from the outside of the cell. This makes them ideal drug targets. With this rational in mind we screened the T. brucei genome for ion channels and ion carriers. Based on this in silico approach we chose six target genes for functional characterization. We showed that down-regulation of three of the targets lead to a severe growth-phenotype in the bloodstream form of the parasites and therefore qualify as potential drug targets. For the functional characterization of the targets we expressed them in Xenopus oocytes and used the 2-electrode voltage-clamp method. We concentrated on two proteins, named TbK1 and TbK2, that showed substantial K+-selective currents at a membrane potential of -40 mV when co-expressed in the oocytes but not when singly expressed. We therefore think the two proteins together form heteromeric potassium channels. Based on the fact that expression in oocytes leads to hyperpolarization of the resting potential in the oocytes we suspect that these channels may be involved in the formation and/or regulation of the plasma membrane potential of the parasites. To check this hypothesis we designed flow cytometry experiments to measure the membrane potential of the parasites with the help of potentiometric fluorescent probes. The results of these experiments indicate a potassium conductance over the plasma membrane mediated by TbK1 and TbK2 in the bloodstream form of T. brucei. Furthermore we could show that these channels are blocked by sub-milimolar concentrations of Ba2+-ions.
Based on these findings we are developing a high throughput assay to search for inhibitors of these channels that could be used as lead-compounds in drug development against the human African Trypanosomiasis.

119 VSG allelic exclusion through transcript interference in African trypanosomes

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Antigenic variation in African trypanosomes is dependent on allelic exclusion among telomeric variant surface glycoprotein (VSG) genes, and involves reversible silencing of other telomeric VSGs. The active VSG transcript is the most abundant polyadenylated transcript in the cell, accounting for approximately 5% of total mRNA. VSG alignments extending through the C-terminal coding region and 3´UTR show substantial sequence similarity and our hypothesis is that VSG allelic exclusion involves interference among these sequences. We demonstrated that RNAi against VSG mRNA triggered VSG switching in approximately 1/500 cells. In contrast, blocking translation of the VSG mRNA using an MS2 RNA-binding protein yields VSG switching in only approximately 1/10,000 cells. These results support a role for the VSG mRNA in allelic exclusion but suggest that additional transcripts could also contribute. Telomeric T2AG3-repeat transcripts could fulfill this role and, in support of this hypothesis, RNAi against these transcripts results in multi-VSG expression on the cell-surface. T2AG3 and VSG transcripts presumably act co-operatively to block transcription of other telomeric VSGs. Also consistent with this, a bicistronic reporter cassette with similar 3´UTRs not normally found at subtelomeres displayed interference, and this was dependent upon VEX1, a protein that also controls VSG allelic exclusion (see abstract by Glover et al.). Our results support VSG allelic exclusion through a mechanism involving transcript interference.

120 A complete molecular toolkit for BioID proteomics and the identification of protein-protein interactions in vivo in trypanosomes

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BioID is a recently developed method that biotinylates proteins interacting with, or in close proximity to, another target protein; allowing for their subsequent identification through streptavidin affinity purification and mass spectrometry. It relies on the generation of fusion proteins consisting of a bacterial biotin ligase (BirA*) and the protein under investigation. It has a number of advantages over classical methods such as yeast-2-hybrid (Y2H) and co-immuno precipitation (Co-IP) assays , namely: It does not use exogenous expression systems (as in Y2H), limiting potential protein-folding and post-translational modification problems giving spurious results. The extreme affinity of streptavidin for biotin allows for the purification of the biotinylated proteins under very stringent conditions and strong detergents, increasing the number of proteins solubilized and available for identification by mass spectrometry. Since the biotinylation of interacting proteins is proximity dependent and not reliant on strong binding between proteins, weak, transient or condition-dependent interactions can be detected in addition to stable complexes. Here we describe a series of plasmid constructs and cloning strategies streamlining the generation of BirA*-target protein fusions. The constructs allow for N- or C-terminal fusions through endogenous tagging methodology, using either classical cloning techniques or, to increase throughput for multiple targets, by PCR-Only Tagging (POT). In order to aid the functional validation of the BirA* fusion proteins, a further series of constructs was created to allow inducible expression of the fusions in cell lines expressing the Tet-repressor system. Optimisation of the biotinylation steps in bloodstream form trypanosomes will also be presented.

121 Identification of a novel Transcriptional Activator Factor that positively regulates VSG transcription in bloodstream trypanosomes

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Antigenic variation in Trypanosoma brucei involves complex genetic mechanisms to exchange the expression of different Variant Surface Glycoprotein (VSG) genes. The VSG gene is mono-allelically transcribed by RNA polymerase I (RNA pol I) from one out of 15 different telomeric loci, known as VSG-expression sites (VSG-ES). The active VSG-ES locus is recruited to a single nuclear body named ESB (Expression Site Body), which contains proteins post-transcriptionally modified by the Small Ubiquitin MOdifier (SUMO) peptide (Lopez-Farfan et al. PLoS Pathogens 2014). Thus, SUMO-conjugated proteins were identified by LC-MS/MS analysis using extracts obtained from bloodstream form trypanosomes that constitutively expressed HA-tagged SUMO. Two independent proteomic screenings led to the identification of a transcriptional activator factor (TAF1) containing a SNF domain that has been previously associated with altering chromatin accessibility in other eukaryotes. To investigate the function of this factor we first developed a monoclonal antibody against TAF1 which detected a single band by Western blot analysis using trypanosome extracts and localized the protein to the nucleus by IF analysis. We next analyzed the effect of TAF1 depletion on gene expression after confirming reduction of TAF1 protein levels. RT-qPCR analysis of TAF1-depleted cells showed a significant reduction of VSG221 mRNA levels, but an increase in Procyclin mRNA, which is repressed in the bloodstream form. However, no significant changes in other analyzed pol I-, pol II- or pol III-transcribed RNAs were detected. Furthermore, ChIP analysis indicated that TAF1 is present in both the active VSG-ES and in the repressed Procyclin chromatin. These data suggest that TAF1 may function as either repressor or activator factor dependent on the respective developmentally-regulated transcribed loci.

122 TbPNT1 is essential for cell viability and kinetoplast maintenance in Trypanosoma brucei

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To date, cysteine peptidases of the Clostripain family (Clan CD, family C11) have only been identified in bacteria, archaea and plants. However, following the elucidation of the first crystal structure of a Clan CD, family C11 protein, PmC11, from Parabacteroides merdae (PDB ID: 3UWS, JCSG), we identified an orthologue in Trypanosoma bru cei , TbPNT1 (Puf Nine target 1; Tb927.11.6550). TbPNT1 is a kinetoplast-specific protein [PMID: 19714224]. Knockdown of TbPNT1 in the bloodstream form by RNAi was lethal and the induced population accumulated akinetoplastic (1N0K) cells. However, RNAi knockdown of TbPNT1 in an akinetoplastic cell line resulted in viable parasites, indicating a role for TbPNT1 in kinetoplast maintenance but no other essential functions in the mitochondrion. Expression of a recoded gene in the TbPNT1 RNAi cell line restored cell viability, whilst re-expression of a recoded mutant lacking the putative active site cysteine failed to restore cell viability after RNAi induction. These data indicate that cysteine peptidase activity is essential for TbPNT1 function. Unfortunately, we were unable to purify soluble TbPNT1 protein for activity assays, so we analysed PmC11. The catalytic His/Cys dyad was identified, along with a potential acidic S 1 binding pocket containing a highly conserved Asp and a site for intramolecular auto-catalytic cleavage. Active recombinant PmC11 had specificity for basic substrates, Arg or Lys as predicted. In summary, our data suggest that an active TbPNT1 cysteine peptidase is essential for cell viability and is exclusively required for kinetoplast maintenance. Importantly, as there are no homologs of TbPNT1 in mammalian cells, TbPNT1 is a potential drug target. This work is supported by the MRC and JCSG is supported by NIGMS GM094586.

123 TriTrypDB: The Functional Genomics Resource for Kinetoplastids

Harb, Omar (University of Pennsylvania)*; Hertz-Fowler, Christiane (University of Liverpool); Roos, David (University of Pennsylvania); Silva, Fatima (University of Liverpool)*

Omar S. Harb, Christiane Hertz-Fowler, David S. Roos, Fatima Silva … on behalf of the Kinetoplastid Database Consortium (TriTrypDB.org) 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 seeks to support and expedite discovery and translational research on the kinetoplastida. Supported data types include the sequences and annotation for 24 species (36 strains), transcript level information (SAGE-tags, ESTs, microarrays, RNA-seq), protein expression data, epigenomic data (ChIP-chip, ChIP-seq), population-level and isolate data (SNPs), and functional information from genome-wide RNAi knock-down analysis. In addition, genomic analyses provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (Enzyme Commission annotations and GO terms), and evolutionary relationships based on gene orthology. Highlights include: A whole genome annotation pipeline (abstract by Steinbiss et al), and community annotation and curation via User Comments, including images, files, PubMed records, etc, are immediately visible through GeneDB and incorporated into TriTrypDB. A semi-graphical Search Strategy system enabling users to construct complex searches in a step-wise manner. Strategies may be saved, modified and shared. For example, see: http://tritrypdb.org/tritrypdb/im.do?s=f6c4cbf89b843e8a Statistical tools enabling analysis of query results for enrichment of Gene Ontology (GO) annotations, metabolic pathways and text terms. KEGG metabolic pathways. New features anticipated over the coming months include better handling of metabolic pathways (including integration of TrypanoCyc and LeishCyc, along with metabolomic datasets), implementation of a workspace enabling users to upload and analyze their own data (e.g. RNA-seq results), and improved functionality of the host response database http://HostDB.org.

POSTERS: Session B
Monday 7:00pm

04/27/2015

125 In vivo RITseq screening for drug targets in the Trypanosoma brucei kinome

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Protein kinases (PKs) are key signaling proteins in eukaryotes and a promising source for druggable targets in Trypanosoma brucei , with several families over-represented with respect to the human host and likely to play parasite-specific functions. In order to assess which PKs are essential for parasite proliferation in vivo , a collection of 183 individual bloodstream form 2T1 T. brucei inducible RNAi lines, representing the whole kinome (JPMID:24453978), were pooled and a modified RNAi target sequencing (RITseq) approach used to assess loss of fitness in a 3 day mouse model of infection. As a result, 56 RNAi-induced lines were identified that had a significant loss of fitness in vivo , 6 of which had not previously been reported to have a loss of fitness in vitro . One of these was a SRPK-like PK involved in regulating DNA repair mechanisms, while the strongest only- in vivo phenotype was caused by depletion of CK2 alpha , a nucleolar PK that is involved in epigenetic regulation in other organisms. One PK found to be essential both in vivo and in vitro was R epressor of d ifferentiation k inase 2 (RDK2). Recombinant RDK2 had protein kinase activity, which was abolished in a K70M mutant. Depletion of RDK2 by RNAi, triggered differentiation from bloodstream to procyclic form prior to cell death (JPMID:24453978). Expression of recoded RDK2 in the RNAi cell line restored cell viability and blocked differentiation, whilst re-expression of a recoded inactive K70M mutant failed to revert the RNAi phenotype. This indicates that protein kinase activity is essential for RDK2 function. Over-expression of RDK2 blocked differentiation induced by cis-aconitate/temperature shift. The role of RDK2 in regulating trypanosome differentiation will be discussed.

126 The dihydroxyacetonephosphate acyltransferase TbDAT is essential for normal growth and synthesis of ether glycerolipids in Trypanosoma brucei procyclic forms

***Zufferey, Rachel (St John's University)**; Pirani, Karim (Kansas State University); Dahlstrom, Kelly (Kansas State University); Cheung-See-Kit, Melanie (St John's University); Seerattan, Elizabeth (St John's Univeristy); Williams, Tyler (St John's University)*

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 in the cell beyond their structural role in membranes; they serve as second messengers in signal transduction pathways, regulate membrane trafficking, and control cell cycle progression. Two enzymes initiate the de novo glycerolipid biosynthetic pathways in T. brucei, the glycerol-3-phosphate acyltransferase TbGAT and the dihydroxyacetonephosphate acyltransferase TbDAT. The present work reports on the characterization of TbDAT that catalyses the first step in ether and ester glycerolipid biosynthesis. TbDAT restored normal growth of and production of wild-type form of the ether lipid based lipophosphoglycan in Leishmania major null mutant lacking endogenous dihydroxyacetonephosphate acyltransferase activity. A conditional null mutant of TbDAT in T. brucei procyclics was created and characterized. TbDAT was important for growth, survival during stationary phase, and synthesis of all ether lipids and ester phosphatidylinositol. However, it was dispensable for GPI-anchored procyclin production. Our results suggest that TbDAT but not TbGAT is the physiologically relevant initial acyltransferase in T. brucei procyclic forms; ii) lipid precursors for phosphatidylinositol production are primarily made by TbDAT, and iii) both TbGAT and TbDAT contribute to the biosynthesis of the lipid moiety for GPI-anchor formation.

127 A novel PRMT heteromer in *Trypanosoma brucei*

Kafkova, Lucie (University at Buffalo); *Fisk, John (University at Buffalo); Lott, Kaylen (University at Buffalo); Zhu, Lu (University at Buffalo); Read, Laurie (University at Buffalo)*

Arginine methylation is a widespread post-translational modification (PTM) that changes its substrates' shape and hydrogen bonding potential, as well as the deposition of other PTMs. It is catalyzed by protein arginine methyltransferases (PRMTs), categorized into three types. Type I and II PRMTs catalyze formation of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine, respectively. Both of these types can also create monomethylarginine (MMA), which is a final product of type III PRMT activity. The focus of our work lies in PRMT function and regulation in Trypanosoma brucei . The T. brucei genome encodes five putative PRMTs, four of which we characterized. TbPRMT1 and 6 display type I activity, and TbPRMT5 and 7 display type II and III activity, respectively. The remaining enzyme has not exhibited any in vitro activity to date; therefore, its name TbPRMT3 is based on homology to human enzyme. Our recent data show a functional interplay between T. brucei PRMTs. The loss of TbPRMT1 leads to decreased abundance of ADMA, while simultaneously massively increasing the number of proteins containing MMA. In dual knockdown studies, we showed that TbPRMT7 scavenges substrates in the absence of TbPRMT1, suggesting potential competition between or regulation of type I and type III PRMT activity on these substrates. To our surprise, we observed the same phenomenon upon downregulation of TbPRMT3, an enzyme we considered inactive. We subsequently discovered that TbPRMT1 and TbPRMT3 form a heteromer that stabilizes both proteins in vivo . Our current focus is aimed at testing a hypothesis that TbPRMT3 is an inactive PRMT homolog that activates TbPRMT1. This scenario would adhere to the recently described enzyme-prozyme paradigm.

128 A functional domain analysis of TbCC2D

Shen, Qian (National University of Singapore)*; Zhou, Qing (University of Texas Medical School); He, Cynthia (National University of Singapore)*

A functional domain analysis of TbCC2D Shen Qian 1 , Zhou Qing 2 and Cynthia Y. He 1 1 Department of Biological Sciencs, National University of Singapore, Singapore 2 Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, Texas, United States of America Flagellum attachment zone (FAZ) is an essential structure that is unique to trypanosomes. It consists of protein filaments, a microtubule quartet (MtQ) in close association with endoplasmic reticulum (ER), both subtending the single flagellum in the parasite. In addition to mediating flagellum-cell body attachment, FAZ also plays crucial roles in positioning the cytokinesis cleavage furrow, hence regulating cell division. In our recent studies, Tb CC2D , a coiled-coil- and C2-domain-containing protein is identified to be an integral FAZ component, essential for FAZ assembly [Zhou et al, 2011] . This protein exhibits a unique bipartite localization on both the FAZ filaments and the FAZ-associated ER. Depletion of Tb CC2D leads to inhibition of new FAZ formation, disrupted organelle segregation and malformed subpellicular microtubules. Bioinformatics analysis of Tb CC2D predicts four coiled-coils in the N-terminal region followed by a C2 domain near the C-terminus. Here we present a functional study of Tb CC2D by looking into individual domains of this protein. We show that the C-terminal C2 domain is dispensable for correct targeting, while at least one of the four N-terminal coiled coils is required and sufficient for FAZ localization. Possible association of the C2 domain with cellular membranes is also investigated. The results suggest that Tb CC2D may act as a linker of FAZ skeletal structure and FAZ-ER. Reference Q. Zhou et al, A coiled-coil- and C2-domain-containing protein is required for FAZ assembly and cell morphology in Trypanosoma brucei. Journal of cell science 124, 3848, 2011.

129 TimX, a novel player in protein import across the inner mitochondrial membrane of T. brucei

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Mitochondrial protein import is an essential function of the unique mitochondrion in T. brucei as roughly 1000 different nuclear encoded proteins need to be correctly localized to their mitochondrial subcompartment. For this reason the responsible import machinery is expected to be similarly complex as in other Eukaryotes. This was recently demonstrated for the translocation machinery in the outer mitochondrial membrane. In contrast, the composition of the inner membrane import machinery and the exact molecular pathway(s) taken by various substrates are still ill-defined. To elucidate this further, we performed a pulldown analysis of epitope tagged TbTim17 in combination with quantitative mass spectrometry. By this we identified novel components of the mitochondrial import machinery in trypanosomes. One of these, TimX, is an essential mitochondrial membrane protein of 42 kDa that is unique to kinetoplastids. This protein migrates on Blue Native PAGE in a high molecular weight complex similar to TbTim17. Ablation of either of the two proteins leads to a destabilization of the complex containing the other protein. Furthermore, its involvement in protein import could be demonstrated by in vivo and in vitro protein import assays. This corroborates that TimX together with TbTim17 forms a protein import complex in the inner mitochondrial membrane. As TbTim17 the TimX protein was subjected to pulldown analysis in combination with quantitative mass spectrometry. The overlap of candidates defined by these two sets of IPs likely defines further components of the inner membrane translocase which are presently being analyzed. In summary our study on novel components of the trypanosome mitochondrial protein import system gives us fascinating new insights into evolution of the mitochondrion.

130 Genome-wide and protein kinase-focused RNAi screens reveal novel pathways of genome repair in Trypanosoma brucei

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Repair of DNA lesions is critical for accurate genome replication and transmission. Many DNA repair pathways have evolved to tackle the wide range of lesions that can arise, and DNA repair pathway choice and activity is frequently closely linked to the cell cycle, ensuring timely lesion removal by the most appropriate route. In the African trypanosome, Trypanosoma brucei , the machinery of DNA repair has been characterised to date through genetic analysis of candidate genes, identified mainly through homology with other organisms, meaning it remains possible that parasite-specific reactions have been overlooked. We have used RITseq to screen genome-wide for T. brucei genes that are important for growth specifically in the presence of the alkylator methyl methanesulphonate (MMS), the first such use of a negative RNAi screen in T. brucei . Several genes and pathways predicted to act in repair but not to date examined in T. brucei were revealed, as well as many novel genes, some that are T. brucei or kinetoplastid-specific. By targeted RNAi and by a further RITseq screen focused on the T. brucei kinome (collection of protein kinases, PKs), we have validated the genome-wide screen and have revealed at least seven PKs that act in the T. brucei response to MMS damage. Amongst these PKs, five have never been implicated in DNA repair in any organism and one is essential only in vivo .

131 An RNAi library screen reveals distinct parasite factors involved in the intracellular trafficking of apolipoprotein L1, the trypanolytic factor in human serum.

Currier, Rachel (London School of Hygiene and Tropical Medicine)*; MacLeod, Annette (University of Glasgow); Alsford, Sam (London School of Hygiene and Tropical Medicine)*

Human trypanolytic factor (TLF) and its lytic component, APOL1, are potent anti-trypanosomal agents, rendering Trypanosoma brucei brucei non-infectious to humans. Following TLF uptake into parasites, APOL1 is transported through the endosomal network and inserted into the lysosomal membrane where it forms pores responsible for osmotic swelling and cell lysis. A genome-scale, loss-of-function RNAi library screen in T. b. brucei selecting for reduced sensitivity to recombinant APOL1 identified a cohort of proteins which may be involved in the trafficking of APOL1. These are distinct from those identified in a previous screen carried out in our laboratory using normal human serum, and includes five subunits of the V-ATPase complex, previously shown to influence TLF efficacy, likely through its role in driving endosomal acidification. Surprisingly, our screen did not identify ICP; T. b. brucei lacking ICP are less sensitive to lysis by human serum, an effect wholly due to the up-regulation of the cysteine peptidase, CATL, in icp null cells, leading to the hypothesis that CATL targets APOL1 for degradation. However, modulation of cysteine peptidase activity using FMK024 (a CATB/L inhibitor) or RNAi against CATL in icp null T. b. brucei confirmed that CATL has no detectable effect on APOL1 efficacy, validating the absence of ICP from our screen outputs. We are currently exploring the influence on APOL1 efficacy and intracellular transit of several novel hits from our screen, including a motor kinesin, which demonstrates a 4-5 fold decrease in APOL1 sensitivity following knockdown, a putative ubiquitin E3 ligase and a putative chloride channel protein.

132 Characterization of novel components involved in host adaptation in Trypanosoma brucei

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Trypanosomes shuttle between a mammalian host and an insect vector during their complex life cycle. In order to survive in these very different environments, the parasite has to adapt its gene expression, morphology, metabolism and organelle activity. To learn more about these adaptation processes, we compared the proteomes of two life cycle stages by a SILAC-based (Stable Isotope Labeling of Amino acids In Cell Culture) proteomics study (Butter et al., 2013). Large-scale proteomic experiments are extremely useful to approach cellular changes in a systematic way. However, to fully understand the requirements for host adaptation of trypanosomes detailed work on a molecular level is necessary. To this aim, we started to characterize highly up-regulated proteins in the human-infective form of the parasite that are also conserved in other trypanosomes. The results of a functional analysis of a protein named p24 will be presented. Using a polyclonal antibody specific for p24, we show that p24 localizes to the flagellum in bloodstream forms (BSF). This is to our knowledge the first flagellar protein described, which is highly up-regulated in BSF. The N-terminal sequence of p24 contains a conserved motif, which appears to be responsible for the association of p24 with the flagellar membrane. p24 has a 37% sequence identity to flabarin, another flagellar protein, which is up-regulated in the insect form of trypanosomes suggesting p24 might be a functional homolog of flabarin in BSF. Co-immunoprecipitation experiments revealed interactions with PAR1, a component of the paraflagellar rod. The possible function of p24 will be discussed.

133 Characterization of a novel telomere-binding protein in Trypanosoma brucei

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Survival of T. brucei in mammalian hosts is dependent on antigenic variation, which is based on monoallelic expression of the variant surface glycoprotein (VSG) and its periodic switching. The active VSG is always transcribed from one of 15 subtelomeric expression sites (ES) while the remaining ESs are silenced. All the ESs are localized at telomeres and it has become clear that telomeres contribute to antigenic variation. Several telomeric proteins that are involved in antigenic variation, such as tbTRF, tbRAP1 and tbTIF2, have been identified and characterized. However, the exact mechanism of their contribution remains to be elucidated. Learning more about the parasite's telomere biology could provide new insights into the transcriptional control of VSG genes. We used two independent approaches to find more components of the telomeric protein complex in T. brucei. First, a pull-down assay with oligonucleotides containing telomeric repeats and second co-immunoprecipitation in order to find novel tbTRF-interacting proteins. Three telomeric candidates were identified in both experiments, including tbTIF2. Here, we report the characterization of a previously unknown telomere-binding protein that we called p45. p45 was verified as a component of the telomere protein complex by indirect immunofluorescence analysis and reciprocal co-immunoprecipitation, whereby interactions with tbTRF, tbTIF2 and tbRAP1 were confirmed. Although it is not essential for viability, mass spectrometry and western blot analysis showed a 4-fold up-regulation of p45 in bloodstream form trypanosomes (BSF). Therefore, we focused on the analysis of VSG expression regulation and switching rates in p45-depleted BSF. Interestingly, we identified and characterized two additional shorter iso-forms of p45. The regulation and potential function of these iso-forms will be discussed.

134 A leucine aminopeptidase is involved in kinetoplast segregation in PCF Trypanosoma brucei

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Leucine aminopeptidases (LAPs) belong to the M17 family of proteases, proteins known for cleaving N-terminal amino acids of peptides as well displaying a broad range of moonlighting functions. Most LAPs described to date are cytosolic proteins, forming homohexamers for catalytic activity. Trypanosoma brucei bares three LAP homologues: TbLAP3060, TbLAP2470 and TbLAP6590. The proteins were tagged, both endogenously and for overexpression, and their localization was assessed by immunofluorescence. All proteins were found to express in different subcellular localizations: TbLAP3060 is associated to the mitochondria, specifically the kinetoplast (kDNA); TbLAP2470 localizes to the nucleus; and TbLAP6590 displays a cytosolic localization. Depletion by RNAi of all proteins has a moderate to negligible effect on cell viability, which may imply redundancy. However, overexpression of TbLAP3060 causes a growth phenotype, alterations of mitochondrial membrane potential, and mitochondrial disruption after 72hrs of induction. 24 hrs after induced overexpression, an accumulation of 1N0K cells is observed, reaching up to 60% of the population after 96 hrs of induction, after which time point cells cease to grow completely. We conclude that the impairment on mitochondrial DNA segregation and eventual loss of kDNA are caused by the kDNA-binding function of LAP that has never been described before for this family of proteins in any other organism.

135 Condensin depletion increases in situ VSG switching frequency in Trypanosoma brucei

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African trypanosomes avoid the host immune response by periodically changing their main surface antigen, the Variant Surface Glycoprotein (VSG), which allows for persistent parasite infection. We have previously described that cohesin complex is involved in VSG switching regulation, as RNA interference of cohesin subunits leads to an increase in the frequency of new antigenic variants (Landeira et al , JCB. 2009). The condensin complex is structurally similar to the cohesin complex, and functions in the three-dimensional organization of chromosomes, preserving structural integrity of the mitotic chromosomes. In addition, condensin has recently emerged as regulator of chromosome architecture and chromatin compaction in interphase, and is enriched near highly expressed genes. To investigate a possible function in antigenic variation we have functionally characterized condensin in bloodstream form trypanosomes. We developed an antibody against TbSMC4 that confirmed the localization of the complex to the nucleus. Interestingly, we also detected SUMOylation of TbSMC4 and TbCND2, a post-translational modification previously associated with VSG expression (Lopez-Farfan et al . PLoS Pathogens. 2014). Most importantly, RNA interference experiments of condensin subunits showed a dramatic increase of VSG221 switching off events, reaching up to 10% of the population after 72h of depletion, which is even higher than what has been previously detected for cohesin depletion. This analysis was carried out by Immunofluorescence using anti-VSG121 and anti-VSGVO2 antibodies and found that condensin depletion increases the detection of these VSGs on the surface, representing in situ transcriptional activation events of independent telomeric VSG-ESs. These results suggest that condensin complex plays an important role in establishing and/or maintaining the transcriptional state of VSG-ES chromatin.

136 TbGem1 is required for normal mitochondrial morphology

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African trypanosomes possess a unique mitochondrion. The mitochondrial outer membrane (MOM) defines the boundary of the organelle and thus represents the interface for inter-organellar communication and transport processes. The morphology of the mitochondrion differs tremendously between insect- and bloodstream-stage leading to the question as to how this is regulated? We recently raised the protein inventory of the MOM in insect-stage T. brucei (Niemann et al., 2013). The highly conserved miro GTPase Gem1 is the only protein found in all three available MOM proteomes (supergroups Opisthokonta, Excavata, Plantae) that is not involved in transport phenomena. We show here that depletion of TbGem1 in procyclic cells results in a severe mitochondrial morphology phenotype, which, however has no to little effect on growth — even in the absence of glucose. The knockdown seems not to have any effect on the fishnet-like appearance of the ER. Ectopic tagging of TbGem1 was used to show that TbGem1 assembles into a high-molecular mass protein complex, as evident from BN PAGE. SILAC Co-IPs were carried out in an attempt to unravel the TbGem1 interactome. The MS analysis detected only ca. 240 proteins in total, with 23 polypeptides enriched >10 fold. Only three of those were not assigned to the MOM, suggesting that TbGem1 forms an extensive interaction network.

137 Kinetics and routes of Trypanosoma brucei infection in the tsetse fly midgut

***Cren, Christelle (Institut Pasteur)**; Schuster, Sarah (Institut Pasteur); Perrot, Sylvie (Institut Pasteur); Bastin, Philippe (Institut Pasteur); Rotureau, Brice (Institut Pasteur)*

Trypanosoma brucei is exclusively transmitted by tsetse fly bites in sub-Saharan Africa. After a bloodmeal on an infected host, parasites embedded in the digested blood are contained in the peritrophic matrix (PTM). During the digestion process, they rapidly reach the posterior midgut, from where some parasites initiate a complex development that will culminate in the salivary glands after 21 days. From electron micrographs of infected midguts, it has been proposed that trypanosomes were crossing the PTM to the ectoperitrophic space, , in order to migrate towards the anterior midgut. As a consequence, they could re-enter the endoperitrophic space in the proventricular region. To examine this process in details, we monitored the kinetics of fluorescent T. brucei development in Glossina morsitans morsitans daily by using a combination of epithelial cell and PTM markers in dissected organs. We determined the precise parasite localization and density in each sub-region with a high-throughput confocal imaging system and compared the infection profiles according to the time after the infective meal and the salivary gland infection status. We observed that less than 48h were required for the parasites to migrate from the posterior midgut to the proventriculus only 5 days after the infective meal. Strikingly, there was no correlation between the presence of trypanosomes in the ectoperitrophic space and in the salivary glands. Instead, we observed a higher proportion of infected salivary glands when parasites were also present in the endoperitrophic space in the anterior midgut. The importance of parasite development in the ectoperitrophic space and the possible roles of the PTM will be discussed.

138 The cyclical development of Trypanosoma vivax in the tsetse fly

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Trypanosoma vivax is the most prevalent trypanosome species in African cattle. It is transmitted by tsetse flies mostly after cyclical development. The latter lasts between 3 and 9 days after an infective meal and is restricted to the mouthparts where epimastigote parasites attached to the cibarium and labrum linings have been observed as well as free infective metacyclic trypomastigotes in the hypopharynx. Whereas infection rates are usually high in tsetse flies (up to 75%), parasite numbers remain low in the proboscis. Here, we investigated the kinetics of T. vivax development in Glossina morsitans morsitans by serial dissections over one week to unravel the different differentiation and proliferation stages. We found that parasite development initiates in the cibarium and labrum after 3 to 4 days, when trypomastigote cells from the bloodmeal progressively disappear, concomitantly to an increase of the epimastigote population. Since no dividing cells were observed at that time, it is possible that this first differentiation occurs in cells with a single kinetoplast / flagellum complex and a single nucleus (1F1K1N). After 3 days, attached epimastigotes were seen in the cibarium and proboscis in growing numbers, due to their proliferation that was attested by the presence of 2K1N and 2K2N parasites. Strikingly, some asymmetrically dividing cells were also observed in proportions compatible with a continuous production of metacyclic trypomastigotes. These cells were dividing in a way that the anterior daughter was in the trypomastigote situation compared to the posterior sibling that remained epimastigote. It is probable that this asymmetric division takes a crucial part in T. vivax metacyclogenesis, as it is the case in other trypanosomatids.

139 cAMP-mediated cell invasion by Trypanosoma cruzi

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Attachment of infective stages of T. cruzi to the host cell is accompanied by elevation of intracellular cAMP levels in the host cell. It has been shown that cAMP was able to potentiate Ca2+-dependent exocytosis of lysosomes and lysosome-mediated invasion by the parasite. However, the contribution during the process of invasion of specific host cell cAMP effectors, such as PKA and Epac, has not been yet elucidated. Exploiting selective activation/inhibition of PKA, we shown that PKA-dependent signalling had no significant effect on invasion. In contrast, Epac specific activation revealed that cAMP-mediated invasion was induced via exclusive activation of the Epac pathway. In accordance to this observation, pharmacological inhibition of Epac completely abolished cAMP dependent effects on invasion.

140 Citrate metabolism in trypanosomes

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Procyclic Trypanosoma brucei express all enzymes required for a complete tricarboxylic acid cycle (TCA), yet there is no measurable CO 2 production in the cycle. Knock-out mutants of aconitase (ACO), citrate synthase (CS), and mitochondrial isocitrate dehydrogenase (IDHm) are all viable in procyclic culture with and without glucose. Surprisingly, neither CS + IDHm nor ACO + IDHm double KO could be generated, in contrast to a perfectly viable CS + ACO double KO. This suggests that production of either citrate or isocitrate is required for reactions unrelated to oxidative decarboxylation. Citrate quantification in those mutants turned out to be a challenging task and required extensive optimization and methods development. We present an optimized protocol for rapid and robust quenching of very low metabolite levels. GC-MS analysis confirmed citrate accumulation in the ?aco/?aco mutant. Citrate could be raised to toxic levels by simultaneous CS overexpression, and this phenotype was rescued by cytoplasmic-only expression of the dually localized ACO. This provides evidence for citrate transport over the mitochondrial membrane. Surprisingly, a glycosomal IDHg isoform was identified as an important citrate sink by accumulation of citrate in a ?idhg/?idhg mutant. We thus provide evidence for a novel pathway in which tricarboxylic acids from the mitochondrion fuel IDHg for production of reductive NADPH in glycosomes.

141 Functional and Biochemical Analysis of the Trypanosoma brucei GPR89 Homologue as a Candidate Receptor for Environmental Cues

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Trypanosoma brucei has a complex life cycle that necessitates its adaptation to the diverse environments of its mammalian host and insect vector. It has multiple developmental forms that differ in their morphology and metabolism, but the molecular mechanisms by which it perceives and responds to changes in its environment are largely unknown. Interestingly, we have found that the T. brucei genome encodes a homologue of the phylogenetically widespread GPR89 family of putative receptors or channels that have been implicated in G protein signalling in plants and Golgi pH regulation in mammals. This implies a signalling function, although the trypanosome genome lacks other conventional components of G protein signalling pathways. We set out to biochemically and functionally analyse the T. brucei GPR89-like (TbGPCR-L1) protein in order to elucidate its potential role in environmental sensing, establishing that the molecule is surface expressed, developmentally regulated in the bloodstream and able to drive premature differentiation to stumpy forms when overexpressed (refer to the accompanying Abstract). The similarity of its localisation and signalling potential to the plant homologues, abscisic acid receptors GTG1 and GTG2, prompted us to explore the potential of the plant protein to mimic the action of TbGPCRL1, revealing that its overexpression can indeed drive stumpy formation. By expressing the T. brucei GPR89 molecule as a recombinant protein or in cell-based reporter systems, we are looking to identify inhibitors or activators of its function. Our analyses suggest that pharmacological intervention in the function of this protein may have important consequences for parasite virulence or transmission. Accompanying Abstract: The Trypanosoma brucei transmembrane surface protein TbGPCR-L1 induces stumpy formation through the quorum sensing signalling pathway

142 Highly unorthodox chromosome duplication revealed by mapping origins of DNA replication in Leishmania

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DNA replication is initiated at genomic sites termed origins of replication, which in eukaryotes are designated by the binding of the origin recognition complex (ORC), a key initiator of replication. All eukaryotic nuclear chromosomes examined to date are replicated from multiple origins, which are selected from a larger pool of ORC binding sites and vary in activation timing or strength. Mapping binding of ORC1/CDC6 (an ORC-like initiator) and origin location shows that Trypanosoma brucei nuclear chromosome replication conforms to this paradigm: though origins are more widely spaced than in other eukaryotes, origin number correlates with chromosome size and origins are found at ~20% of core ORC1/CDC6 sites. However, what distinguishes origin-active ORC1/CDC6 sites from inactive sites is unknown. We have now mapped replication origins genome-wide in Leishmania. These data reveal an unprecedented strategy for replication, since only a single origin can be found in each chromosome, irrespective of size. Indeed, single origins are found in two L. mexicana chromosomes that are each syntenic with two L. major chromosomes, showing that origin singularity survives chromosome fusion or fission. All origins fire with equal efficiency, suggesting variation in origin strength is a facet of chromosome origin multiplicity. Like in T. brucei, Leishmania origins localize to the boundaries of the transcription units and, indeed, show substantial location conservation. However, unlike in T. brucei, Leishmania origin-active loci can be clearly distinguished from inactive loci. Overall, the observation of origin singularity in a eukaryote has implications for the evolution of origin multiplicity and associated controls, and for Leishmania genome maintenance.

143 In vitro and in vivo leishmanicidal activity of a cyclopalladated compound against Leishmania amazonensis and its possible inhibitory effect on recombinant Leishmania topoisomerase 1B

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Leishmaniasis is a neglected tropical disease caused by several species of parasites of the genus Leishmania . Since the available drugs have shown to be highly toxic and cases of resistance have emerged, new therapeutic agents are urgently needed. Here we report the evaluation of a synthetic compound containing Pd(II) and N,N -dimethylbenzylamine (DMBA) against Leishmania amazonensis. The chemical complex [Pd(dmba)(μ-NCO)] 2 (1) exhi bited leishmanicidal activity in vitro against promastigote (IC 50 =13.67 ± 1.54 μM) and intracelular amastigote (IC 50 = 9.29 ± 2.08 μM) forms of this parasite. The selectivity index based on comparison with BALB/c macrophages was 12.16 indicating that (1) presents high selectivity for Leishmania sp. versus mammalian cells. BALB/c mice, infected at the footpad with L. amazonensis and intraperitoneally treated with 0.35 mg/kg.day of (1) during 30 days after disease development, displayed less footpad swelling when compared to the control group that received 2mg/kg.day of amphotericin B. The parasite load was similar to both groups as determined preliminarily by Leishman-Donovan Units (number of amastigotes per 1000 cell nuclei × tissue weight (g)) showing a reduction of 65-68% compared to the untreated controls. Additionally, (1) did not cause exacerbated loss of weight as observed for mice treated with amphotericin B. The inhibitory potency of (1) on recombinant L. infantum topoisomerase IB was also assessed in vitro showing that (1) prevents the relaxation of supercoiled DNA at submicromolar concentrations which could explain its antileishmanial effect.

144 Development of Bioluminescent Trypanosoma cruzi Parasites expressing NanoLuc luciferase to study Chagas Disease Pathogenesis.

Silberstein, Erica M. (Food and Drug Administration)

Erica Silberstein*, Carylinda Serna* , Stenio Fragoso + and Alain Debrabant* *Division of Emerging and Transfusion Transmitted Diseases, Office of Blood Research and Review, CBER FDA, Silver Spring, MD, USA + Instituto Carlos Chagas, FIOCRUZ, Curitiba, Brazil Trypanosoma cruzi is the etiological agent of Chagas disease (CD), which affects 10 million people in Central and South America. CD acute phase occurs shortly after an initial infection , and is followed by a chronic stage which causes cardiac and/or gastrointestinal disorders. During the chronic phase, the level of parasitemia in blood is low and parasites are thought to remain in different tissues. The mechanisms that determine parasite tropism and pathological outcomes are poorly understood. Real-time bioluminescence imaging constitutes a non-invasive powerful tool to monitor pathogen dissemination and tissue distribution within infected animals. To better study chronic CD progression and host-parasite interactions in vivo , we developed transgenic parasites expressing the NanoLuc™ (NLuc) luciferase reporter . This novel small, ATP independent luciferase variant is a very stable 19-kDa protein that possesses ~ 150-fold-greater specific activity than both Renilla and firefly luciferases. Transgenic Colombiana strain parasites were generated after transfection of early log-phase wild-type epimastigotes with a plasmid containing the NLuc gene reporter (pBEX-Nluc). NLuc expressing epimastigotes were obtained by selection in media with G418. Infection of murine 3T3 cells with stationary phase epimastigotes resulted in production of luminescent intracellular amastigotes and release to the culture supernatant of trypomastigotes also expressing Nluc activity, indicating that modified parasites can complete a full life cycle in vitro as the parental wild-type strain. Swiss Webster mice were infected with 5,000 tissue culture-derived trypomastigotes. We plan to perform in vivo imaging studies to define the parameters of the assay and determine the suitability of the NLuc expressing parasites to monitor parasite burden and distribution during the chronic stage of infection.

145 Using a mouse trypanosome to investigate immunomodulatory parasitic nematode gene function in vivo

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We are developing a system using the natural parasite of mice, Trypanosoma musculi , as an in vivo vehicle for the heterologous expression of genes from parasitic nematodes. There is considerable interest in the immunomodulatory function of parasitic nematode genes, but there are a lack of suitable techniques for genetic manipulation, such as RNA interference and transgenesis. We are therefore using a gain of function approach through the heterologous expression of parasitic nematode genes via the extracellular protozoan T. musculi in vivo . We have successfully propagated T. musculi axenically in vitro , and made constructs to incorporate exogenous genes into the parasite genome and target proteins for secretion. T. musculi has been genetically modified to express detectable amounts of green fluorescent protein by incorporation of a construct into the ribosomal RNA gene array, and nematode genes with known or suspected immunomodulatory function are now being investigated. We established a T. musculi line that expresses and secretes Acetylcholinesterase B (AChE B) from the nematode parasite Nippostrongylus brasiliensis . AChE B is hypothesised to neutralise cholinergic immune signalling, and this was tested by characterising and comparing infection profiles of AChE B-expressing T. musculi and control T. musculi in viv o. Acetylcholinesterase activity is detectable in the serum of mice throughout infection with AChE B-expressing T. musculi , but not during a wild-type infection. Expression of AChE B affected the level of parasitaemia, leading to faster clearance of the parasite. To decipher the mechanism and function of this parasite product we are currently analysing local and systemic immune responses, including differential cell recruitment, cytokine expression, antibody development and polarisation of macrophages.

146 Do different African trypanosome species share quorum-sensing signal responses?

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Infections with parasite Trypanosoma brucei progress in waves. Dividing slender form parasites drive the ascending parasitaemia until accumulation of parasite-derived stumpy induction factor (SIF) results in differentiation to growth-arrested stumpy forms. Stumpy forms aid transmission by limiting parasitaemia to prolong infection, whilst also preparing the parasite for development in the tsetse fly vector. Conventionally T. congolense and T. vivax , the major causes of African Animal Trypanosomiasis, are not considered to undergo development in the bloodstream and do not generate morphologically stumpy forms. Here we have analysed the capacity for density dependent arrest in T. congolense and reveal that cell-cycle arrest accompanies peak parasitaemia during infections. Moreover, we have carried out detailed RNA-Seq analysis of the proliferating and arrested bloodstream forms to explore its relationship to the developmental events exhibited by T. brucei . To further examine conservation of the developmental pathways in each trypanosome species we have also tested the ability of T. congolense and T. vivax orthologues of stumpy regulators to complement T. brucei null mutants for these genes. Preliminary data indicates that expression of a T. vivax orthologue can rescue stumpy formation in an otherwise SIF-resistant knockout for the corresponding T. brucei gene . Finally, we have tested the capacity for cross-talk between density sensing signals in different trypanosome species using conditioned medium from T. congolense bloodstream cultures. We demonstrate that this can activate the expression of a stumpy specific reporter protein in T. brucei . Our results highlight the unanticipated capacity for different trypanosome species to exhibit intra and inter specific cell-cell communication in the mammalian bloodstream, with possible consequences for their virulence, transmission and evolution.

147 The cdc2-related kinase 9 (CRK9) forms a unique tripartite complex with a newly identified, L-type cyclin and a kinetoplastid-specific protein

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The silencing of CRK9 , which encodes one of the eleven cyclin-dependent kinases of Trypanosoma brucei, is lethal for procyclic and bloodstream trypanosomes. CRK9 silencing led to a block at the first step of spliced leader (SL) trans -splicing and a loss of phosphorylation of the largest RNA polymerase II subunit RPB1. In addition, we observed hypomethylation of the cap4 structure of the SL RNA which has been implicated in trans splicing. These results identified CRK9 as a key enzyme for an essential, parasite-specific step in the maturation of all mRNAs in trypanosomes. To characterize this enzyme, we generated a cell line that exclusively expressed CRK9 as a fusion with the composite PTP tag and tandem affinity-purified the protein. Subsequent sucrose gradient sedimentation revealed a tripartite complex that was competent in CRK9 autophosphorylation and comprised CRK9, a novel L-type cyclin, termed CYC12, and a protein that is only conserved among kinetoplastid organisms. Since the latter interacted directly with CRK9 we named it CRK9-associated protein (CRK9AP). Silencing either CYC12 or CRK9AP reproduced the CRK9 silencing defects, identifying these proteins as functional partners of CRK9. Additionally, depletion of CRK9AP led to a concomitant loss of CRK9 and CYC12 suggesting that CRK9AP is important for CRK9 complex integrity. Interestingly, the extent of SL RNA accumulation and SL cap hypomethylation upon CRK9 silencing closely paralleled the effects we observed upon interfering with the spliceosomal PRP19 complex, raising the possibility that CRK9 is involved in spliceosome activation. In accordance with this notion, depletion of PRP19 components resulted in the loss of RPB1 phosphorylation, revealing a potential regulatory link between transcription and trans splicing.

148 Mutagenesis of the VSG 3'UTR identifies conserved sequences important for high level expression of VSG in bloodstream form trypanosomes

Ridewood, Sophie L. (Imperial College London)*; Trenaman, Anna (University of Dundee); Hall, Belinda S. (Imperial College London); Ooi, Cher-Pheng (Imperial College London); Rudenko, Gloria (Imperial College London)*

Variant Surface Glycoprotein (VSG) coats the bloodstream form (BSF) T. brucei in a dense layer which protects the cell from the immune system while in the bloodstream of the mammalian host. Extraordinarily, VSG accounts for approximately 10% of the total protein and transcript of the cell, despite being expressed from a single gene. Previous studies using a reporter gene have implicated the VSG 3'UTR as important for differential expression of VSG between the two major life cycle stages. There are two conserved sequence elements (an 8-mer and a 16-mer) in the 3'UTR as well as predicted stem-loop structures formed in many VSG 3'UTRs. We generated VSG 3'UTR mutants to investigate the importance of these features on expression of VSG in BSF T. brucei . Since VSG is essential in BSF trypanosomes, for these analyses, a second VSG (VSG117) was integrated into the VSG221 active expression site. We tested the functionality of VSG117 with mutant 3'UTRs by using RNAi to knock down the endogenous VSG221 mRNA. Cells expressing VSG117 with a scrambled 16-mer were not able to compensate for lack of VSG221, and 5-fold reduction in VSG117 steady state mRNA was observed compared to VSG117 with a WT 3'UTR. This reduction in transcript levels is coupled with a 4-fold reduction in the half-life of the mutated VSG117 transcript and not due to changes in polyadenylation site, as determined by 3'RACE. Therefore the sequence of the conserved 16-mer, but not the 8-mer, is key for high levels of VSG expression in BSF trypanosomes. These data support the hypothesis that there is a RNA-binding factor which stabilises the VSG transcript in BSF T. brucei .

149 Genomic context and VSG expression

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Variant surface glycoprotein (VSG) is the most highly expressed protein in bloodstream form T. brucei . Normally, only one VSG is expressed at a time by RNA Polymerase (Pol) I from 1 of about 15 expression sites (ESs). We investigated the importance of genomic location on VSG expression. It is possible to express an ectopic VSG (VSG117) from the active ES as well as from other Pol I and Pol II-transcribed loci, leading to two different VSG variants expressed on the cell surface. Quantitative protein analyses show that there are at least 10-fold higher VSG levels from actively-transcribed Pol I loci compared with a Pol II-transcribed locus. We also find that the expression level of the endogenous VSG221 is reciprocal to the amount of ectopic VSG117 expressed. This indicates that there is normally a cap on the total amount of VSG that is expressed within a cell. We next compared the effect of the 3'UTR on VSG expression in cell lines expressing ectopic VSG117 from the active ES with either a VSG 3'UTR or an ab tubulin 3'UTR. Transcript levels of ectopic VSG117 with a VSG 3'UTR are approximately 5-fold higher compared to VSG117 with an ab tubulin 3'UTR. This ratio appears to be consistent across different genomic loci. Expression of VSG117 with a chimeric 3'UTR and downstream sequences show that it is the VSG 3'UTR which dictates this effect. These data argue that multiple factors control VSG levels, with high rates of Pol I transcription key for producing sufficient VSG. Additionally, we hypothesise that there is a factor which stabilises the VSG transcript but also limits the total amount of VSG produced.

150 Genome localization of RNA polymerases I and II by ChIP-seq analyses in bloodstream form trypanosomes

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African trypanosomes are protozoan parasites that branched early from the phylogenetic tree of eukaryotes. Trypanosoma brucei shows a combination of unique molecular and cellular features, in particular in the context of transcriptional and genomic organization. Previous work has suggested that transcription might start in Strand Switch Regions (SSRs), however SSRs are often large and it is not clear whether or how transcription starts in SSRs. To address this question we first generated an affinity-purified antiserum against the largest subunit of pol II (TbRPB1), which detected the protein by Western blot analysis and localized RNA pol II in the nucleus by immunofluorescence (IF) analysis. Double 3D-IF deconvolution analysis using anti-TbRPB1 and anti-TbRPA1, the largest subunits of RNA pol II and I respectively, revealed a speckled pattern in the nucleoplasm for RNA pol II, and showed a lack of significant overlap between the two polymerases. To investigate the occupancy of RNA polymerases in the chromatin, we performed a series of chromatin immunoprecipitation combined with massive parallel sequencing (ChIP-Seq) assays. ChIP-seq reads using the anti-TbRPA1 antibody (RNA pol I) were highly enriched at the well-known ribosomal DNA loci and VSG-ES. Using the anti-TbRPB1 antiserum ChIP-seq results revealed a significant accumulation of RNA pol II in 208 peaks along the genome, most of them associated within SSR sequences described previously. The high resolution of this technique allowed us to accurately delimit regions where RNA pol II is accumulated. The peaks had a median size of 190 bases and appeared to be uniformly distributed between convergent, divergent and head-to-tail SSR. The potential of these regions as promoters is being evaluated by analyzing activity of reporter genes driven by these sequences.

151 Towards new drugs for trypanosomatid diseases based on specific high-affinity inhibitors for Trypanosoma brucei kinetoplastid RNA editing ligase 1

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Messenger RNA editing by uridylyl insertion/deletion is a unique process in kinetoplastid mitochondria and therefore a potential drug target. We previously showed that knock-down of RNA editing ligase 1 (REL1), an essential component of ~20S editosome (also called RNA editing core complex, RECC), is lethal in Trypanosoma brucei (Schnaufer et al. , 2001) . REL1 is highly conserved throughout trypanosomatids, which, together with what is known about mitochondrial function in these organisms, suggests an essential function in other pathogens like T. vivax , T. congolense , T. cruzi and Leishmania spp. as well. The crystal structure of the catalytic domain of Tb REL1 (Deng, Schnaufer et al. , 2004) shows a unique active centre with a well-defined ATP binding site. Together with the low sequence and structural similarity between REL1 and DNA ligases (which include the closest mammalian homologs), this suggest the feasibility of developing highly specific REL1 inhibitors with little side effects. We developed a new REL1 activity assay suitable for high-throughput screening (HTS). A proof-of-concept screen against the LOPAC library resulted in a hit rate of 1.7 % and identified interesting REL1 inhibitors such as suramin and the flavonoid myricetin, which has been described as T. brucei hexokinase inhibitor. Another HTS with the Dundee Drug Discovery Unit gave a hit rate of 0.77 % and led to the identification of several promising compound series with potency down to an IC 50 of 20 nM. We are continuing the lead development of the initial hits and will present findings on structure-activity relationships, biophysical characterization, and activity against parasites.

152 Trypanosoma brucei sphingomyelinase enzymes as targets for drug development

Dickie, Emily (University of St Andrews)

My research aim is to investigate Trypanosoma brucei's sphingolipid catabolism as a possible target for novel therapeutics. The T. brucei neutral sphingomyelinase enzyme has already been established as essential to the parasites. This protein has been recombinantly expressed and enriched in E. coli membranes to perform activity-based fragment library screening, using the Amplex UltraRed assay. Fragment hits obtained from this screening have been tested against the parasites for their trypanocidal activity and phenotypic impact in the hope of determining new targeted lead drug compounds. Additionally, "piggy-backing" of several pre-approved human tricyclic drugs, thought to target human sphingomyelinases, has revealed low micromolar trypanocidal activity. The effects of these drugs on parasite morphology, cell-cycle progression and biochemistry are currently being examined. This includes utilizing lipidomic analysis to give insight into how these drugs are affecting overall lipid metabolism and lipid composition. It is hypothesized that the potency of these drugs may be linked to disrupting the activity of a putative lysosomal acidic sphingomyelinase, predicted to be essential to the parasites, as the recycling and processing of scavenged choline–containing lipids is key to parasitic survival. Formation of a conditional knockout of the lysosomal acidic sphingomyelinase is under way to investigate this theory. Localization studies are in progress to establish if this will be one of only a handful of identified lysosomal proteins. Recombinant expression of this membrane protein is also being carried out, which will allow us to assess its acidic sphingomyelinase activity and potential inhibition.

153 Translesion DNA Polymerases and genome maintenance in Trypanosoma brucei

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The genome of every organism is subject to damage, which is tackled either by repair or tolerance. 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), of which five putative examples can be found in T. brucei. We have used RNAi to examine the function of four of these proteins in bloodstream forms of T. brucei . Loss of PolN (Nu) was shown to be severely detrimental to growth, with accumulation of cells showing aberrant nuclei, suggesting a critical role in nuclear genome maintenance. RNAi of PolZ (zeta) did not impair growth, but resulted in increased sensitivity to methyl methanesulphonate (MMS) damage, suggesting a role in the response to alkylation. The sequence of PolQ (theta) suggests that the predicted protein may not be a joint polymerase-helicase like in other eukaryotes, but only a helicase. RNAi revealed that loss of the factor did not affect growth, nor did it result in increased MMS sensitivity. Despite this, we provide evidence that PolQ interacts with BRCA2, a key component of T. brucei homologous recombination and a factor whose loss leads to erosion of the variant surface glycoprotein gene archive. RNAi of PolH (eta) also does not impair growth, and study into the potential contribution of this enzyme to the damage response is ongoing. Taken together, these data reveal widespread and variant functions for four of five putative TLS DNA polymerases in T. brucei genome biology.

154 A new target for a novel class of nitro-heterocyclic-amides with potent trypanocidal activity

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The World Health Organization approved the nifurtimox-eflornithine combination therapy for the treatment of human African trypanosomiasis, renewing interest in nitro-heterocycles therapies for this and associated diseases. We have previously synthesised (J. Med. Chem (2013) 56: 796–806) a series of novel 5-nitro-2-furancarboxylamides that show potent trypanocidal activity, ~1000-fold more potent than nifurtimox against in vitro Trypanosoma brucei with very low cytotoxicity against human cells. Importantly the most potent analogues showed very limited cross-resistance to nifurtimox-resistant cells and vice-versa. This implies that our novel relatively ease to synthesise and therefore cheap, nitro compounds are targeting a different, but still essential, biochemical process to those targeted by nifurtimox or its metabolites in these parasites. Identification of the protein target of these nitro-heterocycles is important, thus the synthesis and use of a number of chemical probes, including fluorescent and photoaffinity tagging allowing target elucidation will be described. A second generation of these analogues has been synthesised to explore SAR even further, and their activity in all three trypanosomes will be presented along with ADMET data. The significant increase in potency over nifurtimox has the potential for greatly reducing unwanted side-effects and also reducing the likelihood of drug-resistance especially in a combinational therapy. Collectively these findings have important implications for the future therapeutic treatments of African sleeping sickness and Chagas' disease. The research leading to these results has also received funding from the European Community's Seventh Framework Programme under grant agreement No.602773 (Project KINDRED).

155 Dissecting the kinome of T. brucei: RIT-seq of cell cycle sorted T. brucei identifies kinases involved in the regulation of nuclear DNA replication

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The coordinated replication and segregation of the genome to daughter cells is an integral cellular process to ensure inheritance and the preservation of life. Several protein kinases (PKs) have been documented to regulate multiple steps in nuclear DNA replication in yeast and other eukaryotes. However, though information is emerging about the machinery and coordination of T. brucei nuclear replication, nothing is known about the putative PK regulation of the reaction. Identification of how nuclear replication is regulated would not only provide insight into the evolution of this essential process, but may open up new avenues for the therapeutic intervention of the diseases caused by T . brucei and other kinetoplasts. To address this, we pooled all bloodstream form T. brucei cell lines that individually target every PK (183 in total) by inducible RNAi (Jones et al., PLOS Pathogens, 2014). The pool was then sorted, with and without RNAi induction, according to their cell cycle stage based on DNA content (G1, S-phase and G2/M) and relative read depth mapped (RITseq) over time and per cell cycle stage. This screen revealed PKs already known to be involved in cell cycle progression (e.g. transition from G1 to S: CRK1 and CRK2), as well as several novel PKs. We are characterizing several of the PKs in detail by examining cellular localization and the effect of their RNAi on DNA synthesis and replication factor localisation. In doing this, we have begun to dissect the PK network involved in T. brucei nuclear DNA replication.

156 Elucidating the target and structure-activity relationships of novel natural product inspired Trypanosoma brucei inhibitors

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Traditional medicinal practices have used natural plant extracts for centuries to effectively treat a range of ailments. The isolation of pharmacologically active natural products from these sources provides access to a diverse range of potential drug-like compounds. Chamuvarinin, of the acetogenin family, was isolated from the roots of Uvaria chamae , an annonaceous plant, which is commonly used in Nigerian folk medicine. As part of our total synthesis endeavour we found synthetic chamuvarinin to have low micromolar activity against cancer cell lines and towards both procyclic and bloodstream form Trypanosoma brucei . The inhibition of complex I of the electron transport chain by acetogenins has been established as their primary mode of action in mammalian cell lines. However, we were intrigued by the low micromolar activity towards bloodstream T. brucei in which complex I is not functional or essential for survival. We will report on the trypanocidal activity and selectivity of a series of highly simplified natural-product like analogues, based upon molecular modelling. Both 1,4-triazole and 3,5-isoxazole substituted heterocycles maintain the low micromolar activity of chamuvarinin, with some increasing the selectivity towards T. brucei compared to mammalian cell lines. Using these analogues, structure-activity relationship studies have identified the key stereochemical motifs of the molecule that are essential to maintain potent activity towards the parasite. Identification of the trypanosomal target is of immediate importance and through the application of fluorescent and photoaffinity tagging of our lead triazole analogues we have synthesized a number of chemical probes for covalent protein modification and target elucidation.

157 Assessing Inosine Monophosphate Dehydrogenase as a drug target in Trypanosoma brucei

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Trypanosoma brucei parasites are incapable of de novo purine biosynthesis and are solely reliant on purine-salvage for growth. In the standard HMI9 medium used for culturing African trypanosomes, hypoxanthine is the predominant form of salvageable purine supplement. This purine derivative is first converted to inosine monophosphate (IMP), an important precursor for the generation of guanosine and adenosine triphophosphates (GTP and ATP). Inosine monophosphate dehydrogenase (IMPDH), which converts IMP to xanthosine monophosphate, is a critical enzyme which commits the fate of IMP towards the formation of GTP. This enzyme was shown by others to be the primary target of mycophenolic acid (MPA) and in T. brucei , gene-amplification of IMPDH was responsible for parasite-resistance to MPA. Consequently, the inhibition of T. brucei cell-growth by MPA also chemically validated IMPDH as a putative drug-target for the treatment of sleeping sickness. In the current study, we have assessed the essentiality of IMPDH in T. brucei using the classical gene knockout approach. IMPDH null parasites (DKO) were initially generated in the presence of various purine supplementations in vitro . However, purine supplementation was found to be redundant in subsequent growth analyses, suggesting sufficient purines were present in calf serum to support growth in vitro . DKO parasites were completely resistant to MPA, but were more sensitive to growth-inhibition by various purines. To determine if serum-purine content in the mammalian host were also sufficient to support growth, mice were infected with DKO cells. Surprisingly, DKO parasites were as infectious as wild-type T. brucei , indicating that IMPDH is non-essential both in vitro and in vivo .

158 Synchronous expression of individual metacyclic VSG genes in Trypanosoma brucei and CITFA binding to metacyclic expression site promoters

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One distinctive feature of the Trypanosoma brucei life cycle is the presence of two populations expressing variant surface glycoproteins (VSGs). Metacyclics in the tsetse fly enable transmission to a new mammalian host, whereas bloodstream trypanosomes must avoid immune destruction to the extent that sufficient numbers are available for transmission when the insect vector takes a blood meal. Here we used our established in vitro differentiation system based on the overexpression of the RNA-binding protein 6 (RBP6), to monitor expression of metacyclic VSGs (mVSGs) during development from procyclics to infectious metacyclic forms. We observed that activation of two mVSGs was simultaneous both at the transcript and protein level, and manifested by the appearance of only a single mVSG per individual cell, confirming allelic exclusion in mVSG expression. The multi-subunit, promoter-binding transcription factor, termed Class I Transcription Factor A (CITFA), was previously shown to be essential for trypanosome viability in culture, indispensable for RNA polymerase I (Pol I) -mediated transcription and directly involved in the regulation of mutually exclusive VSG expression in bloodstream parasites. We found that the CITFA complex also binds the metacyclic expression site promoters in vitro with different affinities. Furthermore, our results suggest that a rapid formation of the mVSG coat is followed by disassembly of the nucleolus, suggestive of overall decrease in transcriptional activity by Pol I in the quiescent metacyclic T. brucei . A model for the activation of mVSG genes that takes into account dramatic changes in transcription will be discussed.

159 Cleavage specificity of the editosome endonucleases in vivo

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RNA editing is an essential post-transcriptional process that creates functional mitochondrial mRNAs in Kinetoplastids. Editosomes are multiprotein complexes that coordinate the catalytic steps of pre-mRNA cleavage, uridine (U) insertion or deletion, and ligation as specified by guide RNA templates. Three compositionally distinct editosomes differ by the mutually exclusive presence of the KREN1, KREN2 or KREN3 endonuclease along with associated partner proteins. These editosomes have distinct substrate cleavage specificities in vitro , but these specificities in vivo are unknown. Because endonuclease cleavage could regulate RNA editing, we sought to elucidate endonuclease specificity in vivo . To circumvent the essentiality of the editing endonucleases, we expressed a mutant gamma ATP synthase allele (MGA) that can alleviate dependence on kDNA expression. Using this MGA approach, we created derivative cell lines from which both alleles of one, two or all three of the endonucleases were deleted, and then examined the effects on in vivo RNA editing and the editosome. Cells lacking endonucleases grew normally, but had altered editosome sedimentation on glycerol gradients. Multiplexed qPCR revealed substantial defects in overall editing. The detailed in vivo specificities of each editosome were revealed by RNAseq analysis. The results indicate that the editosome cleavage specificities observed in vitro are generally recapitulated in vivo , however, in vivo data reveals additional complexities that provide biological insight. For example, KREN2 and KREN3 editosomes have overlapping specificities for some insertion editing sites, but the observed frequency of editing at specific editing sites varied considerably. Thus, endonuclease activities remain distinct despite some functional overlap. These results advance our understanding of how compositionally distinct editosomes collaborate to accurately edit thousands of distinct editing sites in vivo .

160 Investigating the Essential Function of kDNA Polymerase IC in Trypanosoma brucei.

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Replication of procyclic and bloodstream from Trypanosoma brucei kDNA requires at least three essential DNA polymerases (POLIB, POLIC, and POLID) that dynamically localize during the cell cycle. POLIB and POLID have established roles in kDNA maintenance. Single-gene RNAi targeting of POLIB or POLID resulted in a loss of fitness (LOF) and kDNA with an incomplete block in minicircle replication, while simultaneous knockdown of both revealed a complete block in minicircle replication. RNAi of POLIC resulted in LOF and a small kDNA phenotype accompanied by decreased copy number of both minicircles and maxicircles and an accumulation of ancillary DNA, leaving its precise role unclear. Here, we addressed the requirement of POLIC DNA polymerase activity by complementing a POLIC 3'UTR RNAi construct with overexpression of POLIC variants. Importantly, RNAi complementation with WT POLIC rescued the LOF and restored minicircle and maxicircle copy number. In contrast, RNAi complementation with a catalytically-dead POLIC mutant caused a rapid LOF compared to the parental RNAi cell line with no restoration of kDNA copy number, proving that the POLIC polymerase domain is essential. Interestingly, this mutant POLIC also resulted in a 94% decline in maxicircle copy number (4 doublings) compared to the parental RNAi cell line which only had a 70% decline. POLIC is the least abundant of the 3 essential kDNA Pols. To determine if POLIC overexpression could rescue depletion of POLIB or POLID, we generated cell lines that induce RNAi targeting POLIB, POLID, or both, while overexpressing POLIC. Ten-fold overexpression of POLIC was unable to rescue the dual-gene silenced cell line further indicating that POLIC has a unique and specialized role in kDNA maintenance.

161 Glucose-dependent dual localization of the glycosome protein TbPEX13.1

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

162 TbPif1, A mitochondrial helicase with dynamic localization during cell cycle

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Trypanosomatids are defined by the unique catenated organization of their mitochondrial DNA (kDNA). Replication of kDNA is complex requiring a topoisomerase-mediated minicircle release and attachment mechanism while maxicircles replicate within the network. This model indicates a spatial and temporal separation of events. It is predicted that more than 100 proteins are required for kDNA replication and maintenance. Only a subset of these proteins have been identified and characterized. Among these are 4 essential helicases (Pif1, Pif2, Pif5 and Pif8). Trypanosoma brucei Pif2 is essential for kDNA maxicircle replication, while the others have roles in minicircle replication. Our data indicate that spatiotemporal localization of kDNA polymerases is a mechanism to control kDNA replication. To further define the subset of proteins that undergo dynamic localization we are studying the cell cycle localization patterns of TbPif1. Immunofluorescence of PTP-tagged TbPif1 revealed antipodal site localization as previously described. Additionally, TbPif1 has two different localizations during kDNA S phase (1N1Kdiv). At early stages of kDNA synthesis, TbPif1 localized to the kDNA disk, and as replication progresses, it localized at the antipodal sites and co-localized with newly synthesized DNA labeled with EdU. TbPif1 was not detected in 1N1K, 1N2K or 2N2K cells. In contrast to TbPif2, TbPif1 is a stable protein that is not regulated by proteolytic degradation. Posttranscriptional modification, such as acetylation, could be one mechanism that governs TbPif1 dynamic localization. TbPif1 sequence contains several high probability acetylation motifs. We are using recombinant TbPif1 to confirm acetylation events on Pif1. Expression of TbPif1 non-acetylatable mutants will allow us to evaluate the effect of TbPif1 acetylation in vivo.

163 Spatiotemporal localization of the mitochondrial DNA Polymerase IB in Trypanosoma brucei

Rivera, Sylvia (University of Massachusetts, Amhesrt)*; Klingbeil, Michele (University of Massachusetts, Amhesrt); Concepcion-Acevedo, Jeniffer (University of Massachusetts, Amhesrt); Greene, Rebbeca (University of Massachusetts, Amhesrt)*

Trypanosomes contain multiple proteins with non-redundant roles in kDNA replication, some with discrete localization patterns around the kDNA network, mainly in the kinetoflagellar zone (KFZ) and antipodal sites (AS). The current kDNA replication model indicates a spatial and temporal separation of events with early stages (initiation) occurring in the KFZ and later stages (completion of synthesis) at the AS. The spatiotemporal dynamics of kDNA replication proteins and how they coordinate their functions during replication are not well understood. We are investigating the cell-cycle dependent localization of the three essential kDNA polymerases (TbPOLIB, IC and ID) and have established that POLIC and POLID colocalize at the AS with replicating minicircles. POLIC is also detected at the KFZ at early in the replication cycle. Here, we examine the localization pattern of POLIB and demonstrate that POLIB signal is detected near the kDNA disk at all cell cycle stages. The pattern changes during the kDNA replication cycle with POLIB signal appearing in the KFZ and becoming more elongated as replication proceeds, then localizing within the kDNA disk just prior to segregation. However, POLIB never localized to the antipodal sites. Our data on this subset of proteins indicate a highly dynamic environment to coordinate the trafficking of replication proteins during early and late stages of kDNA synthesis. Lastly, we show that silencing of either POLIB or POLID affects POLIC localization and stability, suggesting that recruitment or localization of one kDNA polymerase is dependent on the expression of others.

164 Characterization of an Actin-Binding Protein (profilin) in Trypanosoma cruzi

Osorio Mendez, Juan Felipe (Universidad Nacional Autonoma de Mexico, Instituto de Investigaciones Biomedicas, Departamento de Biologia Molecular y Biotecnologia, Mexico); *Manning, Rebeca (Centro de Investigación y de Estudios Avanzados del IPN, Departamento de Biomedicina Molecular, Mexico); Hernandez, Roberto (Universidad Nacional Autonoma de Mexico, Instituto de Investigaciones Biomedicas, Departamento de Biologia Molecular y Biotecnologia, Mexico); Cevallos, Ana Maria (Universidad Nacional Autonoma de Mexico, Instituto de Investigaciones Biomedicas, Departamento de Biologia Molecular y Biotecnologia, Mexico)*

P { margin-bottom: 0.21cm; direction: ltr; color: rgb(0, 0, 0); }P.western { font-family: "Times New Roman",serif; font-size: 12pt; }P.cjk { font-family: "DejaVu Sans","MS Mincho"; font-size: 12pt; }P.ctl { font-family: "Lohit Hindi","MS Mincho"; font-size: 12pt; } Actin is a major component of the eukaryotic cytoskeleton that is involved in a wide range of cellular processes. One central property of actin is the capability to form polymers, known as microfilaments. The dynamics of formation, structure, and localization of the microfilaments is controlled by a diverse set of actin-binding proteins (ABPs). Typical microfilaments have not been identified in trypanosomatids, but a few genes encoding for putative ABPs, including profilin. Profilin is a structurally conserved eukaryotic protein that binds monomeric actin, promoting its polymerization dynamics by simultaneously interacting with other actin-regulatory proteins. To characterize T. cruzi profilin (Tc-PFN), we immunized mice with purified recombinant Tc-PFN fused with GST and expressed in E. coli . Immunoblot assays using anti-Tc-PFN poly-clonal sera identify a protein of the expected molecular weight in T. cruzi protein extracts from three parasite forms (epimastigotes, and cell culture derived trypomastigotes and amastigotes). In epimastigotes, immunolocalization reveals that Tc-PFN localized predominantly in the cytoplasm as patches at the base of the flagellum and the perinuclear zone. Tc-PFN partially co-localized with actin in these zones, possibly representing regions of active actin polymerization dynamics. Immobilized recombinant Tc-PFN fused to GST co-purified with parasite actin in pull-down experiments. Additional partners that could represent novel actin-regulatory proteins can also be observed in these assays. The presented data demonstrate the expression of Tc-PFN and strongly suggest an interaction with actin and, possibly, with other proteins. We are currently performing experiments to unravel the role of profilin in trypanosomatid actin regulation, and to identify potential novel actin regulatory proteins.

165 Knockdown of IC138 in Trypanosoma brucei: a motility mutant with minor indirect effects.

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Motility in the protozoan parasite Trypanosoma brucei is conferred by the single flagellum, whose motility has some unusual features such as tip-to-base movement and attachment alongside the cell. To understand the regulation of flagellar bending, we have been characterizing TbIC138, the ortholog of a dynein intermediate chain that regulates axonemal inner arm dynein f/I1. TbIC138, was tagged In situ-and shown to fractionate with the inner arm components of the flagellum. RNAi knockdown of TbIC138 results in significantly reduced protein levels, a mild growth defect and a severe motility defect, with nearly 40% of cells immotile by 48 hours post-induction of RNAi. Because cell division and cell cycle defects are often associated with motility mutants, we measured some parameters associated with cell morphology and growth. Slight but significant increases were observed in the incidence of mis-localized kinetoplasts and of cells with nucleus:kinetoplast >1. Flagellar length and cell length were not significantly different between uninduced and 48 hours post-induction. Some flagellar detachment was observed after RNAi induction, but flagellar detachment was not required for abnormal motility and only affected approximately 20% of cells by 48 hours post-induction. The phenotype is consistent with the role of TbIC138 as a regulator of motility, and because changes in motility were observed early on, while indirect phenotypes are minimal, TbIC138 is amenable to mutational analyses for further studies of motility in T. brucei. Flagellar motility is important for pathogenesis and progression through life cycle stages of this parasite.

166 Towards a protein complex map of Trypanosoma brucei

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The functions of the majority of trypanosomatid-specific proteins are unknown, hindering our understanding of the biology and pathogenesis of Trypanosomatidae . While protein-protein interactions are highly informative about protein function, a global map of protein interactions and complexes is still lacking for these important human parasites. Here, benefiting from in-depth biochemical fractionation, we systematically examined the protein complexes of more than 1500 proteins in Trypanosoma brucei , the protozoan parasite responsible for human African trypanosomiasis. Using a rigorous methodology, our analysis led to identification of 69 high-confidence complexes encompassing 510 proteins, including 404 proteins that had no available experimental annotation. These complexes correlate well with known pathways as well as for proteins co-expressed across the T. brucei life cycle, and provide potential functions for a large number of previously uncharacterized proteins. To showcase the utility of this protein complex map, we used it to reveal novel factors involved in the mitochondrial post-transcriptional gene regulatory machinery of T. brucei , and validated them by independent experiments. Interestingly, our results demonstrated that three of our candidates preferentially control the editing process of Cytochrome b (Cyb) transcript, a developmentally regulated mitochondrial mRNA. Our data provide an unprecedented view of the protein complex map of T. brucei , and serve as a reliable resource for further characterization of trypanosomatid proteins.

167 Down regulation of Tim50 in Trypanosoma brucei increases tolerance to oxidative stress

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Trypanosoma brucei, the causative agent for African trypanosomiasis, possesses a non-canonical mitochondrial protein import machinery. We recently characterized a homolog of the translocase of the mitochondrial inner membrane, Tim50, in T. brucei, which possesses a dual-specificity phosphatse activity. TbTim50 knockdown (KD) decreased and its overexpression (OE) increased the mitochondrial membrane potential. TbTim50 OE also increased the production of cellular reactive oxygen species (ROS) and inhibited cell growth. In addition, TbTim50 OE and KD cells showed a different response upon treatment with H2O2. Surprisingly, TbTim50 KD cells showed a greater tolerance to oxidative stress. Further analysis revealed that TbTim50 KD inhibits transition of cells from an early to late apoptotic stage upon exposure to increasing concentrations of H2O2. On the other hand TbTim50 OE caused cells to be in a pro-apoptotic stage and thus they underwent increased cell death upon H2O2 treatment. However, externally added H2O2 similarly increased the levels of cellular ROS and decreased the mitochondrial membrane potential in both cell types, indicating that tolerance to ROS is mediated through induction of the stress-response pathway due to TbTim50 KD. Our preliminary results obtained by a multi-plex iTRAQ (Isobaric tag for Relative and Absolute Quantitation) analyses identified a number of stress and differentiation related proteins those were upregulated in TbTim50 KD and OE cells, respectively. We found the levels of mitochondrial voltage-dependent anion channel (VDAC) and TbTim50 were inversely correlated. Reduction of VDAC along with TbTim50 using a double knockdown parasite, further attenuated the effect of H2O2 treatment on TbTim50 KD cells. Together, these results suggest that mitochondrial proteins and metabolites transporter play a key role in the maintenance of cellular homeostasis in T. brucei.

168 Does the DNA sequence affect nucleosome positioning in T. brucei?

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The dynamic organization of chromatin plays a central role in gene regulation as well as in other essential cellular processes. As the dense packaging of DNA into nucleosomes occludes access to DNA and thereby provides an important level of genome regulation, it is of general interest to understand the mechanisms generating nucleosome positioning. Genome-wide nucleosome mapping revealed a high degree of order in nucleosome positioning in several species. Especially the chromatin organization around transcription start sites (TSSs) is remarkably well-defined. In all species examined thus far a nucleosome-depleted region (NDR) upstream of the TSS and a strong positioning of H2A.Z containing nucleosomes was observed. However, even today it remains unclear how this precise positioning is regulated across the genome. It has been shown that there are favored DNA sequences and that these harbor a 10 nt-periodicity of AT dinucleotides in the minor groove of the nucleosomal DNA. Here we performed genome-wide nucleosome mapping using a high-resolution ChIP-exo protocol followed by paired-end next-generation sequencing in Trypanosoma brucei . To detect the presence of NDRs and DNA sequence preferences of nucleosomes, we isolated canonical nucleosomes as well as H2A.Z containing nucleosomes from TSSs. Our analyses revealed no clear presence of NDRs around TSSs, yet we were able to confirm the already reported nucleosome depletion at the active expression site. Furthermore, we could detect a 10 nt-periodicity of AT dinucleotides in the DNA sequence occupied by histones across the genome. These lines of evidence suggest a DNA sequence dependent nucleosome positioning in T. brucei . An involvement of additional features such as chromatin remodeling complexes, transcription factors and active transcription remains to be investigated.

169 Control of multivesicular body function by PI(3,5)P2 in Trypanosoma brucei

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The mulitvesicular body (MVB) is a specialized late endosome containing intraluminal vesicles (ILVs) that bud internally as early endosomes mature into late endosomes. Ubiquitinated membrane proteins are enriched in ILVs and are therefore targeted for destruction in the lysosome. MVB function and much of the required machinery is conserved in Trypanosoma brucei , though morphological evidence is still lacking. Here, we characterized the role of a signaling lipid, phosphatidylinositol(3,5)bisphosphate (PI(3,5)P 2) in regulating MVB function. Using RNAi, we demonstrated that a PI(3)P 5-Kinase, TbFab1, is essential for T. brucei growth in vitro, and is required for efficient turnover of a transmembrane protein, ISG65. Immunofluorescence analysis of TbFab1-knockdown cells revealed that internalized ISG65 fails to localize to the lysosome, and instead is diffusely distributed throughout the postnuclear region. Many endosomal functions are intact in TbFab1-knockdown cells, including uptake and lysosomal trafficking of soluble cargo, and for biosynthetic trafficking to the lysosome. In yeast, Fab1 kinase function is dependent on its association with a PI(3,5)P 2-phosphatase, Fig4. We have shown that an orthologous protein, TbFig4, is also required for T. brucei growth and ISG65 turnover. Immunofluorescence analysis of a PI(3,5)P 2 biosensor identified the lipid on vesicular structures throughout the cytoplasm, but not colocalized with late endosomes or lysosomes. This prompts questions as to the identity of those structures and their role in the MVB pathway. Together, our results suggest that the regulation of PI(3,5)P 2 and its role in MVB function are conserved in T. brucei . This is in contrast to other mediators of MVB function, such as ESCRT proteins TbVps23 and TbVps4, and may provide clues to the nature of MVBs in T. brucei.

170 Post-transcriptional regulation of Leishmania aquaglyceroporin AQP1

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Leishmania aquaglyceroporin 1 (AQP1) is responsible for important physiological functions such as volume regulation and osmotaxis as well as drug (trivalent antimony, SbIII) sensitivity. However, the mechanism(s) of regulation is largely unknown. In the absence of transcriptional control Leishmania depends on post-transcriptional and/or post-translational control for gene regulation. Recently we found that the cutaneous leishmaniasis (CL) causing species accumulated more antimonite and therefore exhibit higher sensitivity to antimonials, than the visceral leishmaniasis (VL) causing species. This species-specific differential sensitivity to antimonite was directly proportional to the expression levels and stability of AQP1 mRNA. Levels of non-protein thiols, MRPA expression and MRPA activity did not correlate with the antimony sensitivity profiles of the species; suggesting that levels of AQP1 expression determine the sensitivity profiles of the respective species. AQP1 mRNA contains a long 3'UTR (~ 1.8 kb). Regulation of AQP1 mRNA stability was governed by the nature of its 3'UTR. The AQP1 U-rich 3'UTR contains several ARE (AU rich sequences) and CURE (CU rich sequences) motifs that regulate mRNA stability in higher eukaryotes. Structural analysis revealed that the secondary structures of AQP1 mRNA varies widely between CL and VL causing species. ARE and CURE elements of AQP1 mRNA from CL species reside in loop structures, whereas in VL species they reside in stem structures. Roles of different elements of AQP1 mRNA 3'UTR in species-specific regulation of AQP1 will be discussed.

171 Characterization of actin 2, an actin variant of Trypanosoma cruzi.

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Trypanosomatid parasites are characterized by an atypical cytoskeleton mainly composed by microtubules. The presence of a conventional actin similar to the one found in animals and plants have been demonstrated in these parasites but no microfilaments have been detected by electronic microscopy. Interestingly, the bioinformatic analysis of the T. cruzi genome identified the presence of three other genes encoding for putative actins. Of these, Actin 2 (A2) has a 51% identity with conventional actin (A1) and it is only found in T. cruzi but not in T. brucei or Leishmania . Here, we studied the expression of A2 in epimastigotes of T. cruzi. In epimastigotes, the expression of A2 as mRNA was confirmed by PCR using a cDNA library as template. A recombinant GST-A2 fusion protein was generated and used to raise polyclonal antibodies against it. Immunofluorescence assays performed in epimastigote forms with the polyclonal serum showed a speckle pattern all over the parasite, unlike the pattern observed for A1, which is expressed as patches in the flagellum and as a dense concentration near its base. No coimmunolocalization of the proteins detected with these antibodies was observed by confocal microscopy. Preliminary pull-down assays using the recombinant fusion proteins GST-A1 and GST-A2 suggest some differences in the pattern of actin-binding proteins detected for each actin. The interacting proteins will be identified by mass spectrometry. Differences in binding profiles may help to understand the regulation of the actin system of T. cruzi .

172 Thioredoxin-monothiol glutaredoxin hybrid protein from Trypanosoma brucei: biochemistry, structure and biological relevance

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Class-II glutaredoxins (Grxs), also known as monothiol (1-C-) Grxs, are ubiquitous proteins that coordinate iron-sulfur clusters (Fe/S) using the active site cysteines of two monomers and two glutathione molecules. Monodomain 1-C-Grxs are ubiquitous mitochondrial proteins involved in Fe/S biogenesis. Eukaryotes are also endowed with cytosolic fusions of one to three 1-C-Grx domains to a single N-terminal thioredoxin domain. Trx/1-C-Grx hybrids play essential functions in intracellular iron homeostasis and trafficking. Trypanosomatids, causative agents of fatal diseases in human and animals, contain three 1-C-Grxs, with 1-C-Grx3 being a Trx/Grx hybrid. Tb 1-C-Grx3 is expressed both in non-infective and infective stages, and localized at the cytosol. The N-terminal Trx domain harbors a WCxxC motif and is fused to a typical 1-C-Grx domain. The recombinant protein is obtained as a mixture of dimeric holoprotein and monomeric apoprotein. Size exclusion analysis of holo-protein reconstituted in vitro using different low molecular mass thiols suggests ligand-dependent Fe/S binding capacity and conformations of 1-C-Grx3. This protein displays thiol-disulfide oxidoreductase activity albeit moderate compared to Trxs but similar to T. brucei class I (also known as dithiol) Grx. Analysis of 1-C-Grx3 NMR structure shows strong domain-domain interactions with active site conformations that support the biochemical findings (minor oxidoreductase activity and stable Fe/S binding). Additional validation of the data was obtained by the analysis of mutants from charged residues involved in Trx-Grx interaction. In contrast to mitochondrial monodomain 1-C-Grx1, RNAi-mediated depletion of 1-C-Grx3 was not deleterious for the in vitro growth of infective T. brucei . Preliminary animal infection experiments suggest that 1-C-Grx3 is also dispensable for parasite infectivity. The role of 1-C-Grx3 in pathogenic trypanosomatids remains thus elusive.

173 Translational repression by the 3' UTR of the TbZC3H11 mRNA

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Trypanosomatids control their gene expression almost entirely by the post-transcriptional mechanisms. Cis-regulatory elements in the 3' untranslated region (3' UTR) are major determinants of transcript stability and translation and therefore adjust protein levels in response to intrinsic or extrinsic stimuli. We recently showed that ZC3H11, a CCCH zinc finger protein that binds to AU-rich elements within 3' UTR, is required for the procyclic (insect) form heat-shock response. The level of ZC3H11 increases over 10-fold after heat shock, mostly because of increased protein stability and higher translation efficiency. Polysome profiling revealed that under normal culture conditions bulk of the ZC3H11 mRNA co-migrate with small ribosomal subunit suggesting repression of translation initiation via either a block of start codon scanning or impaired large subunit recruitment. However, upon elevated temperatures, mild translational stress from low concentrations of puromycin or MG132 treatment the ZC3H11 mRNA moves to heavy polysomes and its 3' UTR is responsible for this effect. Using deletion analysis of the ZC3H11 3' UTR we mapped a region of 70 nucleotides that was sufficient to restrain translation of the reporter mRNA. We predict that this regulatory RNA sequence is targeted by repressive trans-acting factor that is released upon stress, therefore, relieving translational block of the ZC3H11 mRNA.

174 Distinct phenotypes caused by mutation of MSH2 in trypanosome insect and mammalian life cycle forms are associated with parasite adaptation to cope with oxidative stress

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DNA repair mechanisms are crucial for maintenance of the genome in all organisms, including parasites, where successful infection is dependent both on genomic stability and sequence variation. MSH2 is an early acting, central component of the Mismatch Repair (MMR) pathway, which is responsible for the recognition and correction of base mismatches that occur during DNA replication and recombination. In addition, recent evidence suggests that MSH2 might also play an important but poorly understood role in responding to oxidative damage in both African and American trypanosomes. To investigate the involvement of MMR in the oxidative stress response, null mutants of MSH2 were generated in Trypanosoma brucei procyclic form cells and in Trypanosoma cruzi epimastigote cells. Unexpectedly, the MSH2 null mutants showed increased resistance to H_2O_2 exposure when compared with wild type cells, a phenotype distinct from the previously observed increased sensitivity of T. brucei bloodstream forms MSH2 mutants. Complementation studies indicated that the increased oxidative resistance of procyclic T. brucei was due to adaptation to MSH2 loss. In both parasites loss of MSH2 was shown to result in increased tolerance to alkylation by MNNG and increased accumulation of 8-oxo-guanine in the nuclear and mitochondrial genomes, indicating impaired MMR. In T. cruzi, loss of MSH2 also increases the parasite capacity to survive within host macrophages. Taken together, these results indicate MSH2 displays conserved, dual roles in MMR and in the response to oxidative stress, with loss of the latter function providing life cycle dependent differences in phenotypic outcomes in T. brucei, most likely because of the greater burden of oxidative stress in the insect stage of the parasite.

175 The early-branching trypanosomatid Paratrypanosoma confusum has a complex life cycle

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Paratrypanosoma confusum is a novel uniflagellar monoxenous kinetoplastid discovered in the gut of mosquito Culex pipiens. As the most basal trypanosomatid lineage, it represents a possible evolutionary link between the free-living bodonids and obligatory parasitic trypanosomatids. Paratrypanosoma comes in at least three morphologically distinct stages. The trypomastigote-like swimming stage („swimmer") with a long external flagellum transforms under certain conditions (rich media, basic pH and presence of biopterin) into a „sessile" amastigote-like stage equipped with a short internal flagellum. Moreover, on agar plates, Paratrypanosoma forms an oval stage with a very short external flagellum. Time-lapse video provides evidence of cell division at any of these stages. Preliminary data obtained using antibodies against selected flagellar and cytosolic proteins indicate the existence of possible communication between cells attached to the substrate in the form of a monolayer. RNA-seq data obtained from purified swimmers and sessiles might shed light on molecular background of striking interstagial differences. At least 781 differentially expressed genes indicate higher metabolic activity of the sessiles as compared to the swimmers. Further analysis of the genome of the monoxenous Paratrypanosoma should also provide insight into the emergence of dixenous parasitism of the medically important trypanosomatids.

176 Trypanosoma brucei RAP1 plays an important role in suppressing subtelomeric VSG associated gene conversion by reducing R-loops at telomeres

Nanavaty, Vishal P. (Cleveland State University)*; Pandya, Unnati (Cleveland State University); Li, Bibo (Cleveland State University)*

Trypanosoma brucei is a parasite that causes fatal human African trypanosomiasis. While proliferating inside its mammalian host, T. brucei regularly switches its major surface antigen, VSG, to evade elimination from the host immune response, which is the key virulence mechanism for T. brucei to establish a persistent infection. VSGs are expressed exclusively from subtelomeric VSG expression sites (ESs) in a strictly monoallelic fashion. Both monoallelic expression of VSGs and VSG switching are essential for a successful antigenic variation. We have shown that Tb RAP1, an integral component of the telomere complex, play critical roles in VSG silencing by maintaining a heterochromatic structure at the telomere. Here we show that Tb RAP1 is important for VSG switching regulation. A transient depletion of Tb RAP1 led to an increased VSG switching frequency, and VSG -associated gene conversion is the predominant mechanism. It is well known that actively transcribed regions often form R-loops, which are an RNA:DNA hybrid structure, resulting from invasion of the nascent RNA strand into the double-stranded DNA. It has been shown that R-loops increase DNA double strand breaks and make highly transcribed regions more prone to DNA recombination. In T. brucei , telomeres are transcribed into a long non-coding RNA, TERRA, and depletion of Tb RAP1 leads to derepression of all ESs and elevated TERRA level. We have also observed a stabilization of R-loops at telomeres upon depletion of Tb RAP1, suggesting that in Tb RAP1 depleted cells, elevated TERRA level leads to increased amount of telomeric R-loops and subsequent more frequent DNA recombination at the ES-linked VSG loci, which are immediately adjacent to the telomeric repeats.

177 Targeting Host Cell Kinases As Therapy for Leishmaniasis

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Phagocytic cells such as macrophages must repeatedly engulf Leishmania for this obligate intracellular parasite to survive and cause disease. Therefore, preventing the uptake of Leishmania by macrophages could be a novel therapeutic strategy for leishmaniasis. Amastigotes, the Leishmania life cycle stage found in the human host, bind the Fc receptor and enter macrophages primarily through immunoglobulin-mediated phagocytosis. However, the exact host cell mechanisms that mediate the uptake of amastigotes are poorly understood. We have previously shown that the Arg non-receptor tyrosine kinase facilitates L. amazonensis amastigote uptake by macrophages. Using both small molecule inhibitors and primary macrophages lacking specific Src family kinases, we now demonstrate that the Hck, Fgr, and Lyn kinases also mediate amastigote uptake by macrophages. Arg activation by Src family kinases is required for efficient amastigote uptake. Interestingly, the small molecule combination Arg/Src inhibitor bosutinib, which has recently been approved to treat certain cancers, not only decreases amastigote uptake by macrophages, but also significantly reduces disease severity in Leishmania -infected mice. In particular, there are far fewer parasites contained within the lesions of bosutinib-treated mice than there are within vehicle-treated mice. These results suggest that leishmaniasis could be treated with kinase inhibitors that act on host cells.

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179 A Murine Model of Trypanosome Lytic Factor 2

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Human sterile immunity to infection by Trypanosoma brucei brucei and related species is an effect of the antimicrobial activity of Trypanosome Lytic Factor (TLF). Human serum contains two TLF molecules separable by both structure and function. TLF1 is a 500 kDa mature lipoprotein complex that incorporates apolipoprotein L-1 (APOL1) and haptoglobin-related protein (HPR). In striking contrast, TLF2 is a lipid poor pre-beta 1000 kDa lipoprotein that incorporates both APOL1 and HPR in addition to a natural immunoglobulin M antibody. TLF2 has remained undetectable in the serum of any other TLF1 expressing primate. Additionally, while TLF1 has been reproducibly reconstituted in an experimental mouse model, TLF2 has never been successfully remodeled. Here, we present the initial results from an attempt to create a murine model of TLF2. Mice were generated via hydrodynamic gene delivery of plasmid vectors containing human or primate TLF genes in an activation-induced cytidine deaminase (AID) knock-out mouse background. AID is a critical enzyme involved in the antibody class switching and somatic hypermutation pathways, a knockout of which yields mice incapable of producing fully mature antibodies. Initial findings reveal that coexpression of human APOL1 and HPR is capable of inducing TLF2 formation in this mouse background.

180 You aren't what you don't eat: metabolic specialization is a major driver of genome evolution in kinetoplastids

Seward, Emily (University of Oxford)*; Kelly, Steve (University of Oxford)*

Kinetoplastid parasites occupy a diverse range of host environments, spanning from the bloodstream of giraffes to the fruits of guava. Like many parasitic organisms, these kinetoplastid parasites frequently obtain their energy from a limited pool of host biomolecules. Here we show that metabolic specialization tailored for adaptation to disparate hosts has a broad ranging impact on all aspects of genome evolution. Using bioinformatic approaches we compared genome sequences across this varied group and show that it is possible to detect the signature of metabolic specialization at all levels of gene and genome evolution, including the coding and non-coding regions of the genome as well as at the amino acid level. These findings provide substantial new insights into the evolution of parasitism and the evolution of genes and genome architecture in kinetoplastids. Furthermore they reveal a novel direct link between metabolism and genome evolution that has broad ranging implications for host adaptation. Finally, through the analysis of bacterial parasites, we demonstrate that our findings are generally applicable to other parasitic microorganisms, and thus uncover a previously undetected universal signature of selection.

181 Cyclic AMP binding of a novel signal transducer (CARP1) in T. brucei

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Cyclic AMP (cAMP) is a universal second messenger molecule important in many intracellular signaling processes across all kingdoms of living organisms. Orthologs of cAMP signal-transducing proteins known from other organisms are either absent from the Trypanosoma brucei genome, such as EPAC and cyclic nucleotide-gated ion channels, or are not regulated by cAMP as in the case of a PKA-like kinase. However, an RNAi screen identified a number of genes (termed cAMP Response Proteins or CARPs) that confer resistance to the lethally high cAMP levels produced by inhibition of phosphodiesterases with the drug Cpd A (Gould, et al; 2013). Of the CARPs identified so far, repression by RNAi of CARP1 resulted in the greatest resistance to Cpd A as well as to cell-permeable cAMP analogs. CARP1 consists of a long N-terminus of unknown function and a C-terminus with 3 predicted cNMP-binding pockets. Through affinity purifications, we demonstrate that CARP1 does indeed bind cAMP and competition assays with non-conjugated cAMP give a binding affinity in the low µM range. Also, mutations in the predicted cNMP-binding pockets abolished binding to cAMP. Further characterization of the binding pocket mutants will be presented, as well as confirmatory data on binding specificity. Aside from the phosphodiesterases that degrade cAMP, CARP1 is the only protein in trypanosomes so far to have been shown to bind cAMP. Consequently, we propose that CARP1 is a pivotal cAMP signal-transducing protein in a proposed novel and kinetoplastid-specific cAMP pathway.

182 Quantified markers for the Leishmania lifecycle progression to infectious forms

De Pablos, Luis Miguel (Centre for Immunology and Infection, Department of Biology, University of York, UK.); *Walrad, Pegine (Centre for Immunology and Infection, Department of Biology, University of York, UK.)*

Abstract Kinetoplastid parasites adapt to drastic changes in host environments. This is emphasized during their transmission between insect vectors and human hosts. Leishmania spp. differentiation during host transition is characterised by changes in surface coat, metabolism and morphology, all required for survival in the new host environment. Developmental changes require the parasite cell to interpret changing environmental cues, and to coordinate a swift response for survival. These lifecycle-specific adaptions enable parasite cyclical transmission between hosts, facilitating the persistence of leishmaniasis in human and animal hosts. Developmental events in eukaryotic cells are triggered by signaling cascades in response to environmental cues. Downstream of these signals, specific trans -factors bind mRNAs essential for the cellular response and target them for protein production, storage or degradation. Precise gene regulation coordinates the timely expression of lifecycle stage-specific proteins essential for cell adaptation. In this way, gene regulation promotes parasite survival during host transmission. Here we quantify distinctions between the key lifecycle stages of L.mexicana procyclic and metacyclic promastigotes and amastigotes to demonstrate and outline defining characteristics. We present relative susceptibility to human serum lysis, leukocyte infectivity, cell cycle progression versus quiescence and stage-specific expression of RNA markers. Combined, these present a powerful platform of molecular and cellular markers that will be essential for investigation into Leishmania spp. differentiation. Use of these markers will enable direct, quantifiable comparisons between lifecycle stages in different Leishmania species.

183 Investigating Leishmania development in human: from system analysis to molecular mechanism

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Protozoan parasites of the genus Leishmania are the causative agents of leishmaniasis in humans, cycling between promastigotes in the sand fl y mid-gut and amastigotes in 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, 5% CO 2) has provided new insight into these processes. Transcriptomic and proteomic analyses indicated that differentiation is a coordinated process that results in adaptation to life inside phagolysosomes. Quantitative phosphoproteomics revealed extensive differences in phosphorylation between promastigotes and amastigotes, and identified stage-specific phosphorylation motifs. We used Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) to investigate the dynamics of changes in phosphorylation profile during L. donovani promastigote-to-amastigote differentiation. These experiments revealed protein kinases that phosphorylate specifically by the differentiation signal at the beginning of differentiation, but not by either high temperature or acidic pH alone. The results of these analyses have begun to reveal the molecular basis of differentiation, including a role for protein kinase A (PKA) in its regulation. This work constitutes the first genome-scale interrogation of phosphorylation dynamics in a parasitic protozoa; revealing the outline of a signaling pathway during Leishmania differentiation.

184 TbTIF2 and TbTRF suppress VSG switching through common and independent mechanisms

Jehi, Sanaa (Cleveland State University); *Li, Bibo (Cleveland State University)*

Trypanosoma brucei causes fatal human African trypanosomiasis. T. brucei stays in extracellular spaces in its mammalian host and is constantly exposed to the host immune surveillance. To evade elimination from the host immune response, T. brucei undergoes antigenic variation and regularly switches its major surface antigen, VSG. Antigenic variation is a key pathogenesis mechanism enabling T. brucei to establish long-term infections. There are more than 2,500 VSG genes and pseudogenes in the T. brucei genome, but VSG is exclusively expressed from polycistronic VSG expression sites (ESs) located immediately upstream of the telomeric repeats. VSG switching can occur in an in situ fashion with the coupled silencing of the originally active ES and expression of an originally silent ES. VSG switching can also occur through DNA recombination-mediated events, such as gene conversion and crossover (telomere exchange). We have identified TbTRF as a duplex telomere DNA binding protein and TbTIF2 as a TbTRF-interacting factor. Recently we found that TbTIF2 suppresses VSG switching by maintaining the subtelomere integrity, while the telomere DNA binding activity of TbTRF is also required for suppression of VSG switching. Interestingly, we now find that TbTIF2 is required for maintaining the TbTRF cellular protein level, suggesting that TbTRF and TbTIF2 may function in the same pathway in suppression of VSG switching. However, depletion of TbTRF does not lead to increased DNA double strand break (DSB) amount at the subtelomeric regions as depletion of TbTIF2 does, indicating that TbTIF2 and TbTRF have both overlapping and independent roles in VSG switching regulation.

185 Investigating Machine Learning Methods to Characterize Origins of DNA Replication in Kinetoplastid Genomes

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Origins of DNA replication in the genomes of Trypanosoma brucei and Leishmania species are not well characterized at the sequence level. They lack readily identifiable motifs using conventional sequence-similarity methods; similarities that may obfuscated by the G- or GC-richness of the strand switch regions (SSR) in which they are located. However, it is essential to investigate this sequence further to increase our understanding of the DNA replication in Kinetoplastids and also provide more detailed comparative analysis that may highlight potential mechanisms of genome plasticity in Leishmania species. We are using machine learning techniques, such as k-means clustering and support vector machines to identify the sequence features that may underpin the correct classification of origin and non-origin sequence. Although it may not be possible to determine a specific motif, the inter-species conservation of locations of the origins of DNA replication in Trypanosoma brucei and Leishmania species indicates that there is something specific related to these sequences that is required for a SSR to act as an origin. Machine learning approaches can be used to provide biologically relevant classification of origins of DNA replication and predict these origins in new species or strains. Furthermore, the analysis can be expanded to compare regions of genome plasticity in the Leishmania genus and perhaps understand the relationship between this plasticity and genome replication.

186 New approach to chemotherapy: Drug-induced differentiation leads to lysis of African trypanosomes

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Trypanosoma brucei spp. change their morphology, metabolism and their surface coat during differentiation from bloodstream to procyclic forms. These parasite-specific changes, especially the changes in the surface coat, can be exploited as a drug target. The protein coat of bloodstream parasites consists of variant surface glycoproteins (VSG). During differentiation to procyclic forms in the insect host they lose VSG and replace it by procyclin proteins. While VSG protects parasites from lysis by mammalian serum components, procyclins do not. Inducing premature differentiation from bloodstream to procyclic forms in a patient would therefore be lethal for the parasite. With a transgenic trypanosome line with a reporter gene in a procyclin locus, which is only expressed in procyclic forms, we were able to establish a reporter gene whole-cell assay. This was used to screen for differentiation-inducing compounds in a medium- and a high- throughput format. The aim of the project is to identify compounds which induce differentiation from bloodstream to procyclic trypanosomes at 37°C. Such molecules could serve as new scaffolds for medicinal chemistry to develop a new treatment for sleeping sickness patients. These molecules may not be trypanocidal per se but after loss of the VSG coat, the parasites would undergo rapid lysis within the mammalian host. We will describe the screening approach and our first hits that initiated differentiation in vitro at concentrations 20,000 fold lower than cis-aconitate, a known differentiation inducer.

187 Genetic interaction between base J and other chromatin factors

Kim, Hee-Sook (The Rockefeller University); *Schulz, Danae; Cross, George; Papavasiliou, Nina*

RNA polymerase II transcription initiation and termination regions contain a kinetoplastid-specific DNA modification, known as base J (ß-D-glucosyl-hydroxymethyluracil). It arises via two steps that modify dT: JBP1 and JBP2 (homologs of the mammalian TET proteins) mediated hydroxylation of thymidine to generate a hydroxymethyl-dU (hmU) intermediate, followed by the glucosylation of hmU by the J-associated glucosyl transferase (JGT). Base J synthesis requires both JBP1/2 and JGT. Base J is enriched at regions flanking polycistronic transcription units (PTUs) in T. brucei and in L. major. While T. brucei cells that lack base J are viable, loss of base J in L. major results in rapid death, likely through improper repression leading to transcriptional readthrough at neighboring PTUs and the concomitant accumulation of small RNA species. Since the Leishmania genome does not appear to contain H3V, H4V, and RNAi machinery, T. brucei likely utilizes an additional chromatin marks or RNAi pathways for proper transcriptional termination. Borders of PTUs are also sites for replication initiation, indicating that it is essential to coordinate transcription and replication at these sites. Base J is also found at telomeres. To explore hypothesis that multiple pathways including base J interchange to properly control vital cellular processes, potentially at PTU borders and telomeres, we studied genetic interaction of base J with various chromatin factors. We generated J-null cells by deleting both JBP1/2 in the bloodstream form trypanosome, then subsequently deleted genes of interest. We will present preliminary genetic interaction data and will discuss potential function of base J in T. brucei and its relevance to gene expression in other organisms.

188 Sphingolipid biosynthesis in kinetoplastids.

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Sphingolipids are long-chain bases and their acylated derivatives. The central metabolite in the synthesis of complex sphingolipids is ceramide. Multipass transmembrane enzymes known as sphingolipid synthases (SLSs) transfer polar head groups from phosphoglycerolipids donors onto ceramide, generating phosphosphingolipids. We have previously characterized four distinct sphingolipid synthases (TbSLS1-4) in Trypanosoma brucei (Sevova et al, 2010). TbSLS1 (Tb927.9.9410) produces inositol phosphorylceramide (IPC), TbSLS2 (Tb927.9.9400) produces ethanolamine phosphorylceramide (EPC), and TbSLS3 and TbSLS4 (Tb927.9.9390 and Tb927.9.9380) are bifunctional EPC/sphingomyelin (SM) synthases. T. cruzi and Leishmania have single syntenic IPC synthases. Likewise, T. vivax (at the root of the Salivaria) has a single syntenic SLS of unknown specificity. Using a robust liposome supplemented cell-free expression system coupled with SLS activity assays, we have analyzed the T. vivax SLS (TvY486_0904020), as well as the single syntenic SLS from a free-living organism at the root of kinetoplastids, Bodo saltans (BS87245). Previous work assigned substrate specificity in the T. brucei SLSs to a single dominant amino acid position (aa252) in lumenal Loop V. The presence of Ser at this position confers IPC synthase activity, whereas the presence of Phe confers SM/EPC synthase activity. The product identified in B. saltans possesses a Ser at this position. Experimental analysis confirms that this enzyme is an IPC synthase, reinforcing the model that IPC is ancestral. The enzyme identified in T. vivax, however, has a Tyr residue at this position. We hypothesized that the T. vivax SLS was multifunctional. However, activity assays demonstrate production of SM and EPC only. Our results raise the intriguing possibility that synthesis of IPC may have arisen twice in the evolution of trypanosomatids.

189 The putative CorA homolog of Trypanosoma brucei is not a magnesium transporter

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Magnesium is the most abundant divalent cation in eukaryotic cells. With its involvement in key processes, most importantly as cofactor in numerous enzymatic processes, magnesium plays a central role in cell homeostasis. To date, the magnesium transporters of Trypanosoma brucei remain unknown. Given the importance of magnesium, blocking transport would likely be fatal to the cell. Therefore, knowing the transporters would be a first step to the development of novel drugs to fight human African trypanosomiasis and related diseases such as Nagana. We have identified a putative homolog of the bacterial, or mitochondrial, magnesium transporter CorA in the T. brucei genome. To elucidate the protein's role, we both overexpressed the gene and silenced it by RNA interference. Neither approach affected the growth rate, magnesium requirement or the susceptibility to toxic cations. C-terminally tagged versions (using green fluorescent protein or a hemagglutinin tag) of the Cor A homolog localized to the endoplasmatic reticulum, in accordance with previous reports of Cor A homologs in Leishmania major . Expression of the protein in yeast or in Xenopus oocytes did not result in magnesium transport. We conclude that the Cor A homolog in T. brucei is not likely a homolog of the E. coli protein and therefore must have a novel function within Trypanosoma brucei .

190 An Investigation into the (2-aminoethyl)phosphonate Pathway in Trypanosoma cruzi

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One potential drug target against the intracellular parasite Trypanosoma cruzi (causative agent of American Trypanosomiasis) is the biosynthesis of the glycosylphosphatidylinositol (GPI) anchors and GPI-like molecules. The phosphatidylinositol containing glycosylinositolphospholipids (GIPLs) are the major surface constituent of T. cruzi at all stages of its complex lifecycle. T. cruzi GPI-anchors are uniquely decorated with an unusual (2-aminoethyl)phosphonate (AEP) moiety on the 6-hydroxyl of the glucosamine, however its specific role in host infection and persistence is unknown. Additionally, AEP can also substitute for phosphoethanolamine in the linkage between the GPI anchor and T. cruzi mucins. The AEP containing GIPLs from T. cruzi have been shown to act as virulence factors, likewise, the AEP moiety has been demonstrated to be a virulence factor in a number of bacteria , including the human pathogen Bacteroides fragilis. AEP is entirely absent in Leishmania spp, the closely related Trypanosoma brucei and in higher eukaryotes including humans . Thus, a greater understanding of the poorly characterized AEP pathway may enable the development of parasite-specific inhibitors leading to novel chemotherapeutics in the amelioration of American Trypanosomiasis. My research focuses on the expression, purification and biochemical characterization of the AEP biosynthetic / biodegradative enzymes through a range of techniques. Furthermore, I will provide genetic evidence that the TcAEP pathway is essential for normal cell growth together with an explanation determined by a range of phenotyping experiments. This includes the discovery of a novel metabolite - the high-energy donor for the addition of 2AEP to the T. cruzi GPI-anchors.

191 Functions of MRB1 complex proteins in kinetoplastid RNA editing illuminated by deep sequencing of partially edited RNA populations

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Uridine insertion/deletion RNA editing is an essential process whereby kinetoplastid mitochondrial mRNAs are altered to their final coding forms through sequential utilization of multiple gRNAs that act as templates. Recent data suggest that the Mitochondrial RNA Binding Complex 1 (MRB1) is the major platform for editing. Here, we address the functions of four MRB1 components using Illumina Deep sequencing to analyze the populations of partially edited sequences of two mRNAs in cells expressing or repressed for specific proteins. Analysis with the Trypanosome RNA Editing Alignment Tool created for this purpose and cross-referencing with existing gRNA datasets provides insight into the roles of these proteins in utilization of specific gRNAs. We previously showed that TbRGG2 affects 3' to 5' editing progression by an unknown mechanism. Here, we show that TbRGG2 does not play a role in gRNA exchange, but rather impacts progression of editing though the first and second gRNAs, with specific sites exhibiting significant pauses in the absence of TbRGG2. In contrast, mRNAs from cells depleted of the gRNA-stabilizing protein, GAP1, exhibit editing pauses almost exclusively at gRNA ends. TbRGG2 depleted cells also display a large increase in mRNAs lacking junction regions, illustrating the crucial role of TbRGG2 in active editing. TbRGG2 binding partners, MRB8180 and MRB8170/4160, also affect the progression of editing through gRNAs. However, locations of significantly exacerbated pause sites and characteristics of junctions suggest that TbRGG2, MRB8180, and MRB8170/4160 have distinct but overlapping functions, some of which may be mRNA specific. This represents the first large-scale deep sequencing analysis of edited RNAs and provides important insights into the functions of MRB1 subcomplexes in the editing process.

192 Too much of a good thing? The curious case of Leishmania sphingosine kinase

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Sphingosine kinase (SK) is a key enzyme in sphingolipid metabolism that phosphorylates sphingoid bases (sphingosine or dihydrosphingosine) into sphingosine-1-phosphate (S-1-P) or dihydrosphingosine-1-phosphate (DHS-1-P). In mammals, S-1-P and DHS-1-P are potent signaling molecules implicated in inflammation, cell growth and tumor genesis. While most organisms possess two isoforms of SK, only a single SK homolog (SKa) is recognizable in Leishmania and Trypanosoma species. In Leishmania major, deletion of SKa leads to catastrophic defects in the promastigote stage, which is mainly caused by the accumulation of toxic sphingoid bases (substrates of SK). Provision of myriocin (an inhibitor of sphingoid base synthesis) could rescue the SKa-null mutants in culture. Although not infective to mice originally, SKa-null mutants regained virulence eventually, suggesting the emergence of compensatory changes. Curiously, complementation of SKa-null mutants with a functional SK completely abolishes their infectivity. In contrast, SK expression in wild type L. major does not affect virulence. These observations raise intriguing questions about SKa’s role in Leishmania infection and its potential as a therapeutic target.

193 The flagellar lipidome of trypanosomes

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The trypanosome ciliary/flagellar membrane has a distinctive composition rich in sterols and glycolipids. The biophysical properties of the eukaryotic flagellum are of special interest because of the broad sensory roles played by this ancient organelle. Trypanosoma brucei, the agent of human African sleeping sickness, is a protozoan parasite containing a single polarized flagellum. The surface membrane of the trypanosome is partitioned into flagellar, flagellar pocket and pellicular membrane domains. While the purpose of the flagellum in motility is self-evident, the sensory function of the flagellum is currently being unraveled. We sought to extend our previous finding of qualitative enrichment of sterols, dually acylated proteins and sphingolipids in the T. brucei flagellum by comparing the flagellar lipidome with the whole cell. Using high resolution ESI-MS preceded by HPLC separation, we found the T. brucei flagellum to be enriched in cholesterol and ergosterol species and depleted in various phospholipid species compared to the rest of the body. These quantitative data are consistent with our previous qualitative results on flagellar lipid composition. Through the ongoing functional characterization of differential lipid distribution in T. brucei membranes, especially in the realm of signal transduction, we hope to gain insight into the molecular determinants involved in flagellar sensory function and trafficking.

194 Functions of pentatricopeptide repeat (PPR) RNA binding proteins in mitochondrial mRNA polyadenylation

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The majority of trypanosomal mitochondrial pre-mRNAs undergo massive uridine insertion/ deletion editing which creates open reading frames. However, pre- and post-editing processing reactions are also essential for producing translation-competent mRNAs. For example, pre-editing mRNA adenylation by KPAP1 poly(A) polymerase stabilizes transcripts that are edited beyond few initial sites. In addition, post-editing adenylation/ uridylation by KPAP1 and RET1 TUTase commits fully-edited mRNA to translation. Temporal separation of these events suggests that a mechanism must exist to prevent premature A/U-tailing and to couple the completion of editing with A/U-tailing. To identify protein factors responsible for mRNA 3' modification and coupling with editing, we built a comprehensive protein interactions network of mRNA polyadenylation, editing and translation complexes. RNAi knockdown, in vivo RNA targeting and in vitro reconstitution studies indicate that pre-mRNA is initially stabilized by binding of a specific PPR protein to G-rich motifs. This factor stimulates poly(A) polymerase activity of KPAP1, but not the A/U-tailing. We also identified a distinct PPR factor that binds to a junction between the mRNA and poly(A) tail and blocks premature A/U-tailing. These findings will be presented in a context of integrating editing and polyadenylation processes with mRNA selection by the ribosome.

195 Acidocalcisome-mediated autophagy in Trypanosoma brucei

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Autophagy is a bulk degradation pathway that clears macromolecules or whole organelles through double-layered membrane-bound autophagosomes. This conserved catabolic process promotes nutrient recycling and eukaryotic cell survival during starvation. All about 34 autophagy related genes (ATG) known to date were characterized in yeast, and studies in mammals have revealed a strong connection between autophagy and human diseases such as cancer and neurodegenarative diseases. In the evolutionarily divergent protozoan parasites that are highly relevant to human and animal diseases, increasing evidence is pointing towards a role of autophagy in infectious life cycle development and cell death. However, little is known about the regulation of autophagy in parasitic protozoa, preventing further attempts targeting this pathway for therapies. Though only about half of the known ATG genes were found in T. brucei, all components of the ATG8 ubiquitination system are present and highly conserved. Our published work on procyclic T. brucei (proliferative form in the midgut of tsetse fly vector) confirmed the presence of a bona fide autophagy pathway triggered upon starvation stress. Furthermore, a previously unappreciated role of acidocalcisomes in autophagosome formation was observed in our recent studies. The acidocalcisome, characterized by acidic pH and large contents of Ca2+ and polyphosphates, is an lysosome-related organelle (LRO) found in T. brucei as well as other protozoan parasites including Plasmodium spp., Toxoplasma gondii and Leishmania spp. Autophagy induction strongly correlated with acidocalcisome acidification; inhibition of acidocalcisome biogenesis also inhibited autophagy. Base on these results, we concluded that in T. brucei, starvation-induced autophagy is regulated by a novel acidocalcisome-related pathway, and acidification of the acidocalcisome by an unknown, V-H+-ATPase and V-PPase-independent mechanism, induces autophagosome formation. Additionally, we found that the amino acids-starvation induced autophagic activity depends on the energy level inside the cells.

196 The catalase – an enzyme selectively present only in monoxenous trypanosomatids

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Catalase is a common heme-containing enzyme that catalyzes conversion of the hydrogen peroxide to water and molecular oxygen, thereby protecting cells from its toxic effects. Catalase has one of the highest turn-over rate of all enzymes and is found in nearly all living organisms that are exposed to oxygen. Surprisingly, catalase is present only in the monoxenous trypanosomatids but absent from all their dixenous kins. We have overexpressed the N-terminally tagged catalase in the procyclic and bloodstream stages of Trypanosoma brucei and in the promastigotes of Leishmania mexicana. The overexpression was verified by qPCR, Western blot analysis and the catalase activity test. Interestingly, catalase is active only in the procyclic stage associated with infection in insects and seems to be losing its activity in the bloodstream stage infective for vertebrates. Catalase is localized in the cytosol where it forms dotted pattern, non-overlapping with the glycosomes. The heme content was analyzed by HPLC and is three times higher in cells expressing catalase, as compared to the wild types. Catalase does not affect the viability of the procyclics, and protects them from hydrogen peroxide, as measured by the Alamar blue assay. On the other hand, catalase seems to be disadvantageous to the bloodstream stage in vitro. We aim to verify our findings in vivo by following the virulence in mice as a final vertebrate host. Interestingly we noticed an independent loss of catalase in apicomplexans parasitizing the blood of vertebrates, in silico, a situation reminiscent of that found in Trypanosoma and Leishmania spp.

197 FPC4: a special guest at the FPC party

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Trypanosoma brucei possesses a flagellum that exits the cell from a membrane invagination called the flagellar pocket (FP), the only site for endo- and exocytosis. A ring-shaped structure, called the flagellar pocket collar (FPC), encloses the FP at the flagellum exit site. BILBO1 is the first FPC protein identified and is essential for the FPC and FP biogenesis. Using a Yeast Two Hybrid (Y2H) screen we identified FPC4, a BILBO1 protein partner. Using an anti-FPC4 antibody and an endogenous FPC4-myc cell line, we confirmed that FPC4 localizes to the FPC with BILBO1. We also demonstrated, by Y2H, that the BILBO1 N-terminal domain interacts with the C-terminal domain of FPC4. RNAi knockdown or over-expression of myc-FPC4 has no impact on cell proliferation, BILBO1 expression, or localization in procyclic cells. However, over-expression of myc-FPC4 lacking the BILBO1 binding domain (?B1BD) leads to severe morphological phenotypes. Further, Myc-FPC4(?B1BD) is targeted to the FPC, whereas myc-B1BD is soluble, which demonstrates that the BILBO1 binding domain is not responsible for targeting to the FPC. Expression of FPC4 in a heterologous system (human cells) suggests that the N-terminal domain binds to microtubules (MT) and in vitro assays confirm that FPC4 is able to interact directly with MT. Our preliminary data suggest that FPC4 could be a FPC-microtubule linker involved in FPC positioning during the cell cycle.

198 Deep proteomic analysis of T. brucei FoF1-ATP synthase reveals unique features

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Membrane-bound F o F 1 -ATP synthases generate ATP and are central enzymes in the energetic metabolism of bacteria and eukaryotic organelles. To expand upon our previous proteomics survey of this remarkable rotary machine, we established various procedures to isolate either the catalytic F 1 -ATPase moiety or the entire F o F 1 -ATP synthase from procyclic stage Trypanosoma brucei . The first approach involves two purification steps - ion exchange followed by gel filtration chromatography. The second strategy relies on a GST-tagged inhibitory peptide (TbIF1) to tightly bind the F o F 1 -ATP synthase, which is subsequently purified using a GST-Trap column. Importantly, both purification methods have been optimized to yield very pure complexes that are currently undergoing structural studies using X-ray crystallography and high-resolution cryo-EM. The purification of functional F 1 -ATPase reveals that it is comprised of all the usual eukaryotic components (aß?de), plus a multicopy subunit p18 that is essential. The silencing of p18 results in dramatic losses of F 1 complexes. Furthermore, we mapped two cleavage sites in the sequence of subunit a, which is split into two fragments in vivo . In addition to the F 1 components, the isolated F o F 1 -ATP synthase contains only two homologous subunits of the eukaryotic F o -moiety: the proton channel subunit c and OSCP. Additional identified subunits have no homology outside the Euglenozoa clade, suggesting that the peripheral stalk and membrane-bound subunits are either extremely divergent or replaced by other proteins. Quantitative mass spectrometry with isotope-labelled standards is being implemented to assess the stoichiometry of p18 and selected F o subunits. Our data are in line with accumulating evidence from other non-model eukaryotes that the compositional diversity of functionally conserved F 1 F o -ATP synthases is significantly higher than previously thought.

199 Functional Analysis of a Novel and Essential Subunit of FoF1-ATP synthase in T. brucei

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The T. brucei F o F 1 -ATP synthase is an essential multisubunit enzyme that synthesizes ATP during the procyclic insect stage (PF), but then needs to function in reverse to hydrolyze ATP in order to maintain the essential mitochondrial (mt) membrane potential (?? m) in the infectious bloodform stage (BF). The composition of the T. brucei F o F 1 -ATP synthase is unique compared to the well conserved F o F 1 -ATP synthases found in metazoan, fungal and plant mitochondria because it contains up to 14 novel subunits in addition to the conserved core subunits. Importantly, these subunits do not possess any obvious homology outside of Euglenozoa. This observation raises key questions regarding the function of these subunits within the parasite's complex. The largest of these novel subunits, ATPaseTb1, is a 46kDa membrane-bound protein that co-localizes with F o F 1 -monomers and multimers, but not with F 1 -ATPase. RNAi silencing of ATPaseTb1 in BF T. brucei quickly leads to a significant growth inhibition, changes in mitochondrial morphology and an increase of the ?? m ; all while the structural integrity of the complex is preserved. Moreover, hyperpolarization of the mt inner membrane is also observed upon ATPaseTb1 RNAi induction in PF cells. Interestingly, a bioinformatics search based on Hidden Markov models revealed a strong homology of ATPaseTb1 to the mt inner membrane protein Mdm38 (Letm1), which has been shown to participate in the biogenesis of respiratory chain complexes and acts as the K + /H + or Ca 2+ /H + antiporter. The involvement of ATPaseTb1 in either i) the assembly of the F o -pore, ii) the regulation of the mt permeability transition pore, or iii) mt ion homeostasis will be discussed.

200 Phenotypic and transcriptomic changes during Leishmania donovani culture adaptation inform on potential mechanisms of virulence attenuation.

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We previously showed important differences in infectivity between Leishmania donovani LD1S2D axenic and hamster-isolated splenic amastigotes caused by loss of virulence factor expression during culture. Both types of amastigotes have evolved for years in different environments thus compromising the identification of factors underlying virulence attenuation. To overcome this limitation we performed phenotypic and transcriptomic analyses of splenic amastigotes and derived promastigotes maintained in vitro for 2, 10 and 20 passages, corresponding to approximately 20, 100 and 220 generations. P2 promastigotes showed a generation time of 21 hours that shortened to 8 hours after 10 passages, suggesting selection of parasites with increased in vitro fitness. In contrast, culture-adaptation caused a strong fitness cost in vivo as promastigotes progressively lost their capacity to establish hamster infection. To gain insight into pathways that may cause this dichotomy, we applied RNAseq analysis on splenic amastigotes and derived promastigotes. We identified increased transcript abundance for 387 genes in amastigotes and 991 genes in promastigotes with a fold change > 2 and a p-value < 0.001, thus establishing for the first time in L. donovani stage-specific transcript profiles using biologically relevant parasites. Following changes in transcript abundance during culture adaptation allowed us to establish 16 clusters of co-regulated genes with distinct profiles, including genes that show progressive reduction (including a peptidyl-prolyl cis-trans isomerase-like protein) or increase of transcript abundance (including a pteridine transporter) that may inform on pathways relevant for virulence attenuation and high-density growth, respectively. In conclusion, our study correlates transcriptomic and phenotypic changes that occur during culture adaptation and reveals a mutual exclusive relationship between in vitro and in vivo growth in L. donovani.

201 Characterising the trypanosomatid lysosome and its essential role in host lipid catabolism

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Kinetoplastid related diseases affect millions of people, representing a huge percentage of the world's communicable disease burden. Current drug treatments are woefully inadequate and there is an urgent need for novel therapeutic targets and 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 endocytosis and degradation of host macromolecules from the mammalian bloodstream to provide vital metabolites essential for its proliferation and survival. The terminal compartment of the endocytic pathway, the lysosome, is central to this macromolecular digestion. However, very little is known about this organelle with only a handful of proteins characterised. To successfully study proteins, metabolites and processes of the T. brucei lysosome, it is clear that this organelle must be isolated. Standard fractionation techniques are ineffective due to the high density of the lysosomes preventing separation from larger organelles. Here we have incubated procyclic T. brucei with a non-digestible macromolecule which accumulates in lysosomes, reducing their buoyant density so they can be easily purified from other cellular material. Sucrose gradient centrifugation produced two buoyant fractions distinct from the dense layer of other organelles. Transmission electron microscopy showed one fraction had electron dense particles, comparable in size and morphology to lysosomes. The other fraction showed regular spherical discrete and clustered particles suggestive of endosomes. Proteomic analysis of the lysosome-like fraction detected known lysosomal markers and other proteins with digestive, structural and transport related functions. We are utilising this approach to investigate lysosomal catabolic processes in detail and test compounds that will disrupt the function of this essential T. brucei organelle.

202 The proteome of T. brucei nuclear periphery granules

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Ribonucleoprotein (RNP) granules can be found in all eukaryotes. They consist of RNA and proteins and are thought to play an important role in posttranscriptional control mechanisms by regulating mRNA fate. One important RNP granule type are germ granules, which regulate gene expression via translational repression or protection of maternal mRNAs. In gonads of adult C. elegans , these granules become perinuclear. We recently discovered a novel type of RNP granules in trypanosomes with similarities to perinuclear germ granules, so called nuclear periphery granules (NPGs). The granules form in response to an inhibition of trans-splicing, when polycistronic precursor RNAs accumulate. Just like perinuclear germ granules, trypanosome NPGs are localized at the cytoplasmic site of the nucleus and depend on active transcription. As a first step towards understanding granule function, we have determined the granule proteome by purifying nuclei with and without granules attached. 131 proteins were significantly enriched in the nuclei with granules, including all nine of the previously known granule proteins. 60% of the detected proteins are involved in mRNA metabolism. Data validation and bioinformatic analyses are in progress. The position of the trypanosome nuclear periphery granules close to the nucleus and their dependency on active transcription and mRNA precursor accumulation implicate a possible role in mRNA quality control. With the proteome in hand, and in combination with high-resolution microscopy techniques (EM, single mRNA FISH) we hope to be able to verify this hypothesis in the future. The similarities between NPGs and perinuclear germ granules implicate a common origin of these two types of granules and we may gain some insight into RNP granule evolution too.

203 Tandem affinity purification of trypanosomal DNA polymerase delta identifies a novel essential subunit

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In eukaryotic cells, accurate and efficient nuclear genome replication is carried out by three essential DNA polymerase complexes: DNA polymerases a-primase, d and e . Little is known about nuclear DNA replication in the Kinetoplastids, and we aim to characterise the three replicative nuclear DNA polymerase complexes from Trypanosoma brucei to identify potential drug targets. Pol d has roles in DNA synthesis, DNA repair and homologous recombination. Human and fission yeast Pol d are heterotetrameric complexes (however budding yeast Pol d is heterotrimeric) consisting of the catalytic PolD1 and accessory subunits PolD2, PolD3 and PolD4. PolD1 and PolD2 are well conserved in T. brucei, however, T. brucei PolD3 and PolD4 homologues could not be identified using similarity searches. Using tandem affinity purification we have purified the Pol d complex from a T. brucei cell line expressing PTP tagged PolD2. TbPolD2 pulled down the catalytic TbPolD1 and a potential novel TbPol d subunit, tentatively referred to here as TbPolD3. TbPolD3 possesses two PCNA binding motifs, a common feature among proteins involved in DNA replication and repair. Furthermore recombinant TbPolD3 can bind recombinant TbPCNA, and this binding is dependant on one of the TbPolD3 PCNA binding motifs. TbPolD3 knockdown using RNAi inhibits procyclic form cell growth, suggesting it is an essential protein in T. brucei. Further analysis of TbPolD3 RNAi and conditional knockout cell lines is being carried out to elucidate the essential functions of TbPolD3. This research is partly funded by MSD and the Scottish Universities Life Sciences Alliance (SULSA). The opinions expressed are those of the authors and do not necessarily represent those of MSD Limited, nor its Affiliates.

204 Understanding Novel Functional Properties and Interactions Associated with a Leishmania Poly-A Binding Protein Homologue (PABP1)

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The Poly-A binding protein (PABP) is a conserved eukaryotic protein involved in many aspects of mRNA metabolism, consisting of an N-terminal RNA binding region, a non-conserved linker segment and its unique C-terminal PABC domain. During protein synthesis, at its initiation stage, PABP facilitates the recruitment of polyadenylated mRNAs by the translation machinery. In model eukaryotes this is accomplished through a direct interaction between PABP and the heterotrimeric initiation complex eIF4F, through its eIF4G subunit. In Leishmania, the PABP1 homologue has been shown to be involved in translation initiation and was also seen to be targeted by phosphorylation events. PABP1 has also been seen to bind to a Leishmania homologue of eIF4E (EIF4E4), another eIF4F subunit, through an interaction unknown from other eukaryotes. Here, we have investigated the PABP1 phosphorylation and its interaction with EIF4E4. Upon transfection into L. infantum cells, phosphorylation of an HA-tagged PABP1 was seen to be directed to multiple serine and threonine residues within the protein's linker region. Mutations in these residues abolished phosphorylation but did not prevent PABP1 from associating to polysomes. For the EIF4E4 interaction we used in vitro GST pulldown assays and found that mutations in three conserved residues within the PABC domain of PABP1 abolished EIF4E4 binding. In vivo, a mutant PABP1 impaired in this interaction was, nevertheless, targeted by phosphorylation and could bind to polysomes. Neither loss of phosphorylation nor EIF4E4 binding could prevent the ability of episomally encoded PABP1 mutants to complement the loss of its endogenous genes. Our results are consistent with this Leishmania PABP homologue being involved in novel interactions and regulation events not yet seen in other eukaryotes.

205 CHARACTERIZATION OF NEW eIF4F SUBUNITS IN Trypanosoma brucei

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In trypanosomatid protozoa the presence of a modified cap4, plus the spliced leader sequence on the 5'end of the mRNAs, and the existence of multiple homologs of Translation Initiation Factors suggests differences in the way mRNA is recruited for translation. In eukaryotes initiation of protein synthesis starts with the binding of the translation initiation complex eIF4F to the mRNA cap. This complex allows the recognition of the mRNAs by the small ribosomal subunit and the initiation of translation. eIF4F consists of three subunits: eIF4A, a DEAD box RNA helicase; eIF4E, the cap binding protein; and eIF4G, a large scaffold protein. Unusually, kinetoplastids possess six eIF4E and five eIF4G and two eIF4A homologs. We have identified three eIF4F complexes associated with TbEIF4E5 and TbEIF4E6 and two putative mRNA cap-generating proteins. Using primer extension and RNAseq experiments on complex-bound mRNAs we have found that these complexes bind mature mRNAs involved in several metabolic pathways. We also found these complexes respond to nutritional stress and methylation inhibitors forming Nuclear Peripheral granules, thought to be involved in mRNA maturation. Through the use of RNAi experiments it was shown that the complex performed by TbEIF4E5- TbEIF4G2- Tb927.11.6010 proteins is not essential for cell survival in culture but is critically involved in cell motility. Combined, the collected data suggests that the complexes formed by TbEIF4E5 and TbEIF4E6 may be involved in specific control of mRNAs.

206 Tim62, a Novel Mitochondrial Protein in Trypanosoma brucei, is Essential for Stability and Assembly of the TbTim17 Protein Complex

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Ujjal K. Singha, VaNae Hamilton, and Minu Chaudhuri Department of Microbiology and Immunology, Meharry Medical College, Nashville, TN 37208 Trypanosoma brucei, the causative agent of Human African trypanosomiasis (HAT), imports hundreds of proteins into its single mitochondrion. However, mitochondrial protein import machinery in trypanosomatids remains enigmatic. Previously, we identified the translocase of mitochondrial inner membrane (TIM) in T. brucei consisting of TbTim17. TbTim17 is associated with several trypanosome-specific proteins those are required for mitochondrial protein import. Here, we further characterized the function of one such protein, TbTim62. TbTim62 possesses an ankyrin repeat motif near the N-terminus and a putative nucleotide-binding motif near the C-terminus. The protein is localized in the mitochondrial inner membrane and its import into mitochondria depends on TbTim17. TbTim62 is present in the mitochondrial protein complexes of ~120 kDa and ~1100 kDa. Whereas, TbTim17 is primarily present in a ~1100kDa complex and in multiple complexes within the range of 300-600 kDa. A minor fraction of TbTim17 was also found in the ~120 kDa complex. Knockdown (KD) of TbTim62 reduced the levels of TbTim17 protein complexes as well as reduced the steady state levels of TbTim17 post transcriptionally. The half-life of TbTim17 was decreased more than 4 fold in TbTim62 KD versus the parental T. brucei. Further analysis revealed that TbTim62 KD did not have any effect on the import of TbTim17 into mitochondria, but it markedly inhibited assembly of TbTim17 into the larger protein complexes. Both TbTim17 and TbTim62 assembles first in a ~120 kDa complex, which is the assembly intermediate of the matured complexes consisting of TbTim17. All together our results showed that TbTim62, is a novel component of the mitochondrial protein import machinery and is critical for stability and assembly of the TIM complex in T. brucei.

207 Identification and functional characterization of a Leishmania major HSP70-related protein with an unusual domain structure

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Previous proteomics investigations of Leishmania donovani promastigotes and axenic amastigotes showed a significant difference in the stage-specific phosphoproteome and revealed the hypothetical protein LinJ.29.1330 as an interesting candidate with potential implication in adaptive stage differentiation. Using multiple sequence alignment, we showed that (i) annotated LinJ.29.1330 lacks 51 amino acids at the N-terminus as judged by proteomics identification of a diagnostic N-terminal peptide, revealing its misannotation in the Leishmania genome databases; (ii) LinJ.29.1330 is present in all Leishmania species an d in ancestral trypanosomatids, but absent in related pathogenic Trypanosoma brucei and T. cruzi ; (iii) although LinJ.29.1330 is closely related to HSP70, it has a very divergent HSP70 domain and an additional TPR-domain involved in protein-protein interaction. Given its unusual domain structure, we termed the protein HSP70-related (HSP70r). The study of the endogenous protein by Western Blot analysis using total protein extracts obtained from parasites at different growth phases showed that the HSP70r level is constant during logarithmic phase but decreases during stationary phase. Moreover, an increase in protein abundance was observed in parasites exposed to 34°C for 24h but not in those exposed to other stress conditions suggesting that HSP70r is a heat shock-regulated protein similar to other HSP70 family members. Finally, structure-function analysis expressing various HSP70r deletion constructs in transgenic parasites revealed that overexpression of the TPR-domain alone caused a significant growth delay during logarithmic phase, which was associated with a high percentage of cell death of up to 40% at day 3 of logarithmic growth. We are currently investigating the mechanisms underlying the dominant negative effect of TPR-domain over-expression that may allow us to gain insight into HSP70r function.

208 Stalled ribosomes and the control of translation in the infective metacyclic trypomastigote forms of Trypanosoma cruzi.

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In Trypanosoma cruzi, gene expression regulation occurs mainly at the posttranscriptional level. Previous studies from our group have shown that mRNAs encoding proteins of the small subunit proceossome complex (SSU), are more abundant in metacyclic trypomastigotes (MT) than in epimastigote (E) stages. However, even though a greater amount of TcSof1, TcImp4 and TcDhr1 mRNA is observed in MTs associated to polysomes, no protein products were observed and no new synthesis was detected. Trapping mRNAs in polysomes is a way to block translation, suggesting a mechanism of translational downregulation, probably at the elongation and termination stages. The aim of our study is to understand gene expression regulation processes involved in the repression of translation in MT forms of T. cruzi. In particular, identify the presence of stalled ribosomes and the sub-population of mRNAs regulated by this mechanism. We monitored the translational profiles in E and MT using RNA-seq and ribosome profiling. Using harringtonine, a drug that binds 60S ribosomes and has been reported to block initiation, followed by ribosome profiling analysis, we observed the presence of ribosome footprints along the coding region of TcSof1, TcImp4, TcDhr1 transcripts in MTs. This result strongly suggests that ribosomes are stalled onto the mRNA. Moreover, several transcripts showed this pattern of footprints along the coding sequence even after harringtonine treatment, with no protein product detected. Gene ontology analysis showed that these co-regulated genes are associated at the functional level. Our results suggest that a functionally related population of transcripts is negatively regulated at the translation level by a mechanism that stalls ribosomes along the mRNA. This work was supported by FIOCRUZ, CNPq, and CAPES (Brazil)

209 The regulation of the mRNA expression by PUF3 could be a mechanism to explain the resistance to benznidazole in trypanosomes

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Proteins with Pumilio RNA binding domains (PUF) are known mainly as posttranscriptional repressors of gene expression that reduce mRNA translation and stability. Trypanosoma cruzi and Trypanosoma brucei have 10 and 11 PUF proteins, respectively. However, just few have been functionally characterized. Evidence, mostly derived from yeast, suggests that PUF3 is involved in translation control of mitochondrial proteins. Benznidazole (Bz) is a pro-drug used to treat Chagas disease. In the parasite, it needs to be activated at the mitochondrial level. Its main mechanism of action might involve a capacity to modulate the oxidative stress response within the parasite. Both processes, drugs activation and oxidative stress response, involve mitochondrial function. In this context, we have sought to characterise the role of the PUF3 expression in T. cruzi and T. brucei, in particular the effect on susceptibility to Bz and hydrogen peroxide. We found that RNAi-mediated down-regulation of TbPUF3 was associated with increased sensitivity to Bz. Consistent with this, over-expression of PUF3 in T. cruzi increased resistance to Bz, and to exogenous to hydrogen peroxide. Interestingly, mRNA levels of mitochondrial proteins, including the Bz-activator TcNTRI, were found to be down regulated in T. cruzi overexpressing PUF3. Taken together, our results support the idea that mechanisms involved in Bz resistance in trypanosomes could be mediated by PUF3.

210 Characterisation of Leishmania casein kinase 1 isoform 2 localization and interaction

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The Leishmania genome encodes for six CK1 isoforms, including LmjF35.1010, which is the only isoform identified in published proteomics studies. Casein kinase 1 isoform 2 (CK1.2) is an ecto-kinase, released from parasites in culture and present in exosomes. We have shown that CK1.2 is (i) the most conserved kinase among Leishmania species suggesting important functions in parasite biology, (ii) the Leishmania kinase that shows the highest identity to its human ortholog, and (iii) essential for the survival of intracellular parasites using a pharmacological approach. Despite its crucial functions in parasite biology and infection, CK1.2 localisation and interactions required for its release via exosomes are unknown. Here, by using transgenic parasites expressing a V5-tagged CK1.2, we show that CK1.2 is localised in the flagella, and shows both diffuse and vesicular localisation in the cytoplasm of promastigotes, whereas CK1.2 has mostly a diffuse cytoplasmic localisation in axenic amastigotes. Using native electrophoresis, we showed that most of CK1.2 is part of high molecular weight complexes in promastigotes, but not in axenic amastigotes. Our preliminary data indicate that in promastigote, CK1.2 could be part of at least three different complexes ranging from 140 to 500 KDa. To establish a first experimental link between localization and interaction, we are currently performing immuno-precipitation and mass spectrometry analysis for the identification of CK1.2 binding partners, which will inform on potential CK1.2 functions. Given the role of the N- and C-terminal domains in regulating localisation of the highly conserved human CK1 homolog, we are currently investigating the importance of these domains for the correct localisation of Leishmania CK1.2 by immunofluorescence using transgenic parasites producing V5-tagged deletion mutant proteins.

211 In search of glycosomal nucleotide sugar transporters

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Trypanosoma brucei have N-glycosylated and GPI anchored glycoproteins that are essential for their survival. N-glycosylation and GPI anchoring utilize glycosyltransferases that require nucleotide sugars. Unlike mammalian cells, nucleotide sugar biosynthesis takes place inside glycosomes (peroxisomes) in T. brucei (1-6). This suggests the existence of a novel family of glycosome membrane nucleotide sugar transporters (NSTs) to allow the transport of nucleotide sugars out of the glycosome, prior to uptake into the Golgi by conventional NSTs (7). To look for candidate glycosomal NSTs, we obtained a high-confidence glycosome proteome using glycosome epitope tagging and SILAC proteomics (8). After excluding known transmembrane proteins, like peroxins, GIM5A, GIM5B and GAT1-3, we selected 22 transmembrane proteins as possible glycosomal NST candidates. Of these, 6 gave an expected RNAi growth phenotype in bloodstream form trypanosomes and, of these, 2 gave VSG glycosylation RNAi phenotypes. Further phenotyping of these putative glycosomal NSTs are under way using nucleotide sugar analysis by mass spectrometry. References: 1. Roper et al. (2005) J Biol Chem 280, 19728-19736 2. Stokes et al. (2008) J Biol Chem 283, 16147-16161 3. Marino et al. (2010) Glycobiology 20, 1619-1630 4. Marino et al. (2011) Eukaryot Cell 10, 985-997 5. Bandini et al. (2012) Mol Microbiol 85, 513-534 6. Kuettel et al. (2012) Mol Microbiol 84, 340-351 7. Liu et al. (2013) J Biol Chem 288, 10599-10615 8. Guther et al. (2014) J Proteome Res 13, 2796-2806

212 Functional Significance of the Evolution and Architecture of the Trypanosome Nuclear Pore Complex

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Much of the core architecture of the eukaryotic cell was established over one billion years ago. However, many cellular systems possess lineage-specific features, and architectural and compositional variation of complexes and pathways is likely keyed to specific functional differences. The nuclear pore complex (NPC) is responsible for many processes including nucleocytoplasmic transport, interactions with the nuclear lamina and mRNA processing. NPC structure and composition are best documented in Saccharomyces cerevisiae and mammals. We exploited trypanosomes to investigate NPC evolution at the level of conservation of protein-protein interactions and composition, allowing us to unambiguously assign NPC components to specific substructures. Thus the NPC structural scaffold is generally conserved, albeit with lineage-specific elements. However, there is significant variation in pore membrane proteins and an absence of critical components involved in mRNA export in fungi and animals. Concomitant with this, we identify features that point to an ancient system for the export of mRNA in trypanosomes. Identification of these lineage-specific features within the trypanosome NPC significantly advances our understanding of the mechanisms of nuclear transport, gene expression and of the evolution of the nucleus.

213 The dynamics of VSG coat replacement during antigenic variation in African trypanosomes

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Trypanosoma brucei is a protozoan parasite whose surface coat consists of a dense and repetitive array of a single Variant Surface Glycoprotein (VSG). Though this VSG coat elicits a robust, antibody-mediated immune response, the organism can evade the immune system by switching the VSG encoding gene that is expressed, resulting in the display of an antigenically distinct surface coat. Because switching occurs at a low frequency in vitro , it has been difficult to directly observe trypanosomes undergoing the process of replacing the “old” VSG coat with a new, distinct coat. Using a genetically modified T. brucei strain, which can be induced to switch at a heightened frequency, we have been able to observe large populations of trypanosomes undergoing VSG coat replacement. We are currently utilizing this system, along with a novel labeling method that allows visualization and tracking of VSGs over time, to investigate the dynamics and mechanisms of coat replacement during switching. We have shown that full loss of the old VSG coat takes greater than 36 hours in vitro , and have unexpectedly discovered a novel mechanism of inheritance of the new VSG coat during cell division.

214 Energy metabolism in different Trypanosoma cruzi lifecycle stages and the impact of host metabolism on intracellular amastigotes.

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During its complex life cycle, the Chagas disease parasite Trypanosoma cruzi transitions between triatomine insect vectors and mammalian hosts. To adapt to these vastly different environments, the parasite must alter its metabolism to changing nutrient conditions. While insect stage epimastigote metabolism has been relatively well characterized, metabolic differences predicted to exist between the mammalian infective stages of T. cruzi—cell-invasive trypomastigotes and intracellular amastigotes—are not well understood. Moreover, there is no information regarding the extent to which intracellular amastigotes are able adapt to host cellular metabolism and perturbations in flux. Here, we exploit a Seahorse XFe24 extracellular metabolic flux analyzer to probe energy metabolism in three main life stages of T. cruzi: epimastigotes, trypomastigotes and amastigotes. We find that amastigotes, isolated from mammalian host cells, utilize exogenous glutamine more efficiently than trypomastigotes and resist nutrient deprivation better than trypomastigotes. In that respect, amastigotes appear to be more robust than trypomastigotes. Next, we sought to determine whether metabolic remodeling can be detected in amastigotes isolated from human fibroblasts that were maintained under conditions designed to promote high rates of glycolysis relative to oxidative phosphorylation and vice versa. T. cruzi amastigotes, isolated from fibroblasts grown under these different conditions for 1 or 2 passages, do not differ in their basal metabolic capacities or in their rates of glucose and glutamine utilization. These findings suggest that stable metabolic reprogramming does not occur in intracellular amastigotes – at least under these particular experimental conditions. Future studies will determine whether intracellular parasites are buffered from changes in host cellular metabolism or whether they maintain flexibility in their ability to adapt to current conditions.

215 An RNAi toolkit for functional genetic analysis of Leishmania (Viannia) braziliensis

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RNA interference (RNAi) is a powerful genetic tool applied in Leishmania braziliensis. (Lye et al PLoS Pathogens 2010). We developed vectors for rapid generation of 'stem-loop' (StL) constructs using Gateway (Invitrogen®) technology and characterized the length of the stem required for efficient knockdown. Unexpectedly, stems whose length is below about 250 nt showed strongly reduced efficacy on reporter or endogenous genes. The need for longer stems raises the likelihood of off-target effects from the long StL constructs, which may be minimized and/or controlled for by various approaches. A second phenomenon noted was that our StL RNAi constructs usually generated high levels of siRNAs, estimated by next-gen sequencing of siRNAs to exceed those for the endogenous SLACS and TATE elements, which usually comprise >80% of siRNAs; moreover in some cases high levels of 'residual' dsRNA could be seen in StL transfectants. The impact of both high siRNAs and dsRNAs on Leishmania biology is not certain and may differ amongst loci and transfectants; these concerns will need to be taken into account in interpreting results. We have used RNAi to study quinonoid-dihydropteridine reductase (QDPR), a challenging locus encoded by multiple tandemly repeated QDPR genes (>99% nucleotide identity) interspersed with two other genes (Lye et al J.Biol. Chem. 2002). Introduction of a 'consensus' 588 nt QDPR StL resulted in a reduction in QDPR activity to 12% of WT, which will allow us to assess the role of QDPR in folate metabolism and antifolate chemotherapy. Lastly, we probed the role of the amastigote induced A600 gene family. As seen in studies of L. mexicana A600 knockouts, RNAi of Lbr A600 results in a strong inhibition of amastigote but not promastigote viability. Thus by transfection of promastigotes or amastigotes followed by differentiation, stage-specific functions can be studied. * Corresponding author: beverley@borcim.wustl.edu

216 A new software resource for rapid automatic annotation of kinetoplastid genomes

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With many efficient and accurate sequencing technologies at the disposal of researchers today, it is becoming more important to quickly produce structural and functional gene annotations of newly sequenced, draft quality genomes. To make full use of the assembled data, an annotation software should provide a comprehensive, high-quality set of both coding and non-coding gene features, as well as information about orthologs in reference genomes, protein domain structure and basic functional information. While such software solutions are available for prokaryotes [1-2], there is still a need for easy-to-use software which specifically takes the requirements of kinetoplastid genomes into account, such as gene structure and genomic organization. We present a full-stack software pipeline to annotate kinetoplastid genomes in various states of assembly, covering all stages from pseudochromosome contiguation, gene model predictions to functional annotation. The pipeline integrates both the transfer of annotations from related species as well as de novo gene finding, with particular support for partial genes likely to be found in highly fragmented assemblies. Gene models are automatically named according to user-provided patterns and annotated with product descriptions, GO terms, orthologs and domain model hits. Special care is taken to produce semantically valid annotation files in standardized formats to reduce the turnaround time to database submission. We demonstrate the use of the pipeline to annotate 22 unpublished Leishmania and Trypanosoma genomes, produced as part of the NHGRI/NIAID Kinetoplastid White Paper Project (9 already available through TriTrypDB), as well as to extend currently available annotations (e.g. T. congolense) with additional functional data. [1]Aziz et al. BMC Genomics, 9:75 (2008) [2]Seemann. Bioinformatics, 30(14):2068-9 (2014)

217 Decoding anti-leishmanial drug efficacy and resistance mechanisms using RNAi library screening in Trypanosoma brucei.

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An improved understanding of mechanisms underlying anti-leishmanial drug modes-of-action and resistance would likely facilitate surveillance and the development of new and improved interventions. We have previously selected a bloodstream-form T. brucei RNAi library with the anti-trypanosomal drugs, identifying T. brucei factors responsible for drug efficacy, including an aquaglyceroporin (AQP2; melarsoprol/pentamidine). We have now carried out similar T. brucei RNAi library selections using the anti-leishmanial drugs: sodium stibogluconate (SSG), miltefosine, amphotericin-B and paromomycin. This has provided insights into drug-uptake, and highlighted candidate Leishmania orthologues that may explain efficacy and resistance mechanisms. T. brucei possesses three AQPs that phylogenetically cluster with LmAQP1, the loss of which is known to render Leishmania less sensitive to SSG. We identified TbAQP3 as a specific SSG transporter, revealing remarkable substrate selectivity amongst the T. brucei AQPs. We also identified a role for a lysosomal Major Facilitator Superfamily Transporter in paromomycin efficacy; this channel also contributes to suramin sensitivity and sensitivity to other aminoglycosides, such as G418. Finally, we identified a group of phospholipid transporters that make a varied contribution to amphotericin-B and miltefosine efficacy, as well as a locus that may influence the efficacy of both drugs. Our findings provide insights into the anti-trypanosomal action of these important drugs, which should facilitate the identification of the resistance mechanisms that undermine the current anti-leishmanial therapies.

218 A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in Leishmania donovani

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The salvage of environmental purines is an essential process for all known parasitic protozoa. Our previous work has demonstrated the ability of Leishmania to sense and adapt to restrictions in their extracellular purine environment. The removal of purines invokes both a major morphological and metabolic remodeling—conferred at the mRNA, protein, and metabolite levels, that allows for survival in the absence of extracellular purines for greater than 100 days in culture. To understand the mechanism of environmental purine sensing, we have used purine salvage pathway mutants, specifically cell lines that cannot convert between AMP and GMP, a process essential for purine homeostasis. By effectively separating these two purine metabolite pools, these mutant cell lines require two exogenous purine sources to provide both adenine and guanine nucleotides necessary for growth and replication. Culturing these mutant parasites in any single purine source resulted in growth arrest, morphological changes, and an increase in the abundance of proteins previously identified in the wild type Leishmania purine stress response—hallmarks of purine starvation. Since a response to purine stress can be induced in this mutant in the presence of any single extracellular purine source, we conclude that purine sensing must occur through the surveillance of perturbations to the intracellular purine environment, rather than by environmental monitoring of the extracellular purine milieu. Significantly, we observed a divergent survival phenotype between independent disruptions of the adenine and guanine nucleotide pools, and our data demonstrate that perturbations in the intracellular levels of adenine nucleotides, rather than guanine nucleotides, are key for inducing the metabolic changes needed for the long-term survival of Leishmania cultured in the absence of purines.

219 A Zinc finger-containing RNA binding protein impacts mitochondrial gene expression in Trypanosoma brucei

***Ammerman, Michelle (Kettering University)**; Volobuev, Denis (Kettering University); Downey, Kurtis (University at Buffalo); Read, Laurie (University at Buffalo)*

RNA editing in the mitochondria of T. brucei is catalyzed by the multiprotein RNA editing core complex (RECC), however, a number of additional complexes and factors are required for efficient editing including the Mitochondrial RNA Binding (MRB) complex. Accessory proteins associate with the RECC and MRB complex in an RNA-dependent manner and connect the complex to RNA regulatory steps beyond editing, such as RNA processing and production, RNA cleavage and translation. MRB6070 is an RNA binding protein that was initially identified as having an RNA-dependent association with the MRB complex. Consistent with the presence of RanBP2 type zinc finger (ZnF) binding domains in the protein, in vitro reactions showed binding of MRB6070 to RNA. RNA interference was used to knockdown MRB6070 expression in procyclic form cells and demonstrated that MRB6070 is essential for cell proliferation. Analysis of mitochondrial gene expression in MRB6070-depleted cells using quantitative PCR did not show an overall effect on RNA editing, but instead showed alterations in the levels of specific RNAs. These results suggest MRB6070 interacts with mitochondrial transcripts in a sequence-specific manner through binding of MRB6070's ZnF domains. Together, these results demonstrate MRB6070 is an essential protein involved in the regulation of mitochondrial gene expression.

220 Identifying transcription termination sites in trypanosoma brucei

Lopes da Rosa-Spiegler, Jessica (UGA)*; Sabatini, Robert (University of Georgia)*

In Eukaryotes, RNA transcription is restricted to individual genes flanked by distinct transcription start sites (TSS) and transcription termination sites (TTS). The TSS delineates the beginning of polymerization, not necessarily the entry point for the RNA Polymerase-DNA template interaction. On the other end, transcription termination requires 3' end processing of nascent RNAs and results in dissociation of polymerases from the template. TSS and TTS are major factors in modulating mRNA levels by ensuring that the necessary un-translated regions (UTRs) for mRNA stability are synthesized and to prevent engaged polymerases from interfering with the expression of downstream genes. Kinetoplastid genomes, however, are organized in long directional gene clusters called polycistronic units (PTUs) with a single TSS and TTS at each end. Transcription initiates at the most upstream non-coding region, often a divergent strand switch region (dSSR). Transcription is continuous across multiple genes and RNA processing signals without dissociation of the polymerase from the template. Termination should therefore occur at the most distal non-coding region termed convergent strand switch regions (cSSR). In Leishmania species, inhibition of base J synthesis leads to global accumulation of RNA species that originate from cSSR, implying that recognition of the TTS is compromised. In order to identify base J-independent cis and trans acting factors involved in TTS recognition, we have designed a reporter assay that detects localized transcription termination in Trypanosoma brucei . Thus far, we show that cSSRs are not sufficient to halt transcription in our assay. However, we have discovered an artificial sequence that leads to early termination and are in the process of further analysis. Additionally, we are expanding our criteria to identify native TTS.

221 Characterization and DNA Sequence Specificity of the Base J Associated Glucosyltransferase

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Trypanosomatids possess a unique DNA modification in their genomes known as base J. Base J is synthesized in a two-step process in which thymidine in DNA is hydroxylated by the thymidine hydroxylases JBP1 and JBP2 forming hydroxymethyluracil (hmU). A glucose moiety is then attached to hmU by a glucosyltransferase (GT), to make base J. Base J is localized at specific places throughout the genome, particularly telomeric repeats and chromosome-internal regions involved in Pol II transcription initiation and termination. Previous studies have suggested that the GT involved in J synthesis is non-specific and will transfer glucose anywhere hmU is present in the genome. Thus, it is presumed the localization of base J is determined by the thymine hydroxylases (JBP1 and JBP2) involved in the first step of the synthesis pathway. We have recently identified the base J-associated glucosyltransferase (JGT) and now show that recombinant protein representing the proposed GT domain is sufficient for in vitro activity. We use a Leishmania episome system to characterize the sequence specificity of J synthesis in vivo; WT telomeric sequence (GGGTTA) supports J synthesis, while various mutations do not (GGTTT, …). Analysis of the same DNA sequences in the in vitro assay indicates the sequence specificity of J synthesis is not at the level of the GT reaction. Additional studies will be presented which define the minimal DNA substrate required for GT activity and specific contacts between the JGT and hmU substrate.

222 Development of biological assays to assist Human African Trypanosomiasis (HAT) drug discovery

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HAT is caused by kinetoplastid parasite Trypanosoma brucei gambiense and is endemic to 36 sub-Saharan African countries, with <10,000 new cases reported every year. The current anti-trypanosomal therapies suffer from problems of toxicity, inadequate efficacy and need advanced clinical settings to treat patients. Hence, there is an urgent need for safer, efficacious and easy to use oral drugs against HAT. In order to identify novel chemical entities to successfully cure HAT, we embarked on large-scale screening of Novartis proprietary compounds. We developed several biological assays to validate and progress different compound series in the drug discovery pipeline. Initially, compounds were subjected to IC 50 determination using ATP as a surrogate marker for viability, followed by cidality testing. Compounds were grouped in to concentration and time dependent kill and time dependent kill using kill kinetics experiments. The time to kill experiments gives insight into the pharmacological parameter that could drive the efficacy of compounds in animal models. Further, we also developed methods to determine absolute concentration required by compounds to kill all parasites completely below limit of detection under in vitro conditions (AC cure). Presence of single living parasite in animals is sufficient for the relapse of disease leading to eventual death. Hence, reaching the in vitro AC cure concentrations in vivo in animal models may eradicate all parasites resulting in complete cure. We have also employed live imaging, light and fluorescent microscopy, FACS based analysis for grouping and prioritizing different compounds series based on the mechanism of action. Multiple biological assays employed will help in expediting the process of selection of high quality chemical matter having different mode of action for further optimization.

223 PATHOGEN MEDIATED EVOLUTION OF APOL1 HAPLOTYPES

Pant, Jyoti (The Graduate Center, City University of New York)

PATHOGEN MEDIATED EVOLUTION OF APOL1 HAPLOTYPES Jyoti Pant1,2, Joey Verdi1,2, Maria Nelson2 and Jayne Raper1,2. 1. The Graduate Center, City University of New York. 2. Hunter College, City University of New York ABSTRACT Apolipoprotein L1 (APOL1) is a primate specific innate immune factor that protects against select Kinetoplastida and some viruses. The APOL1 gene, which arose due to gene duplication, is rapidly evolving and positively selected. In the human population, 16 haplotypes of APOL1 have been detected with five showing geographic specificity and the mark of positive selection. These are named G0-G4 where G0 is present in 80% of the total population, G1, G2 and G3 are positively selected in specific African populations and G4 is the Neanderthal APOL1 present in 22% of modern Europeans and Asians. Although previous research implicated G1 and G2 as being selected against human infective African trypanosomes, selection pressure/s for G3 or G4 have not been studied. We hypothesize that APOL1 in humans is evolving due to pressure from a broad range of pathogens including African Trypanosomes. We aim to test the effect of these five major APOL1 haplotypes on Trypanosome brucei Ssp. and Leishmania sp. in vitro and in vivo. Our data shows that all haplotypes (G0-G4) are active against T. b. brucei and differentially active against T. b. rhodesiense and Leishmania sp.

224 Evolution of Protein Palmitoyl Acyltransferases in Kinetoplastids

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Palmitoylation is a post-translational modification of proteins which occurs only in eukaryotes, either nearby and subsequent to myristoylation of the N-terminal glycine residue or elsewhere in the protein. This modification stabilizes the association of proteins with distinct cellular membranes and, in some cases, allows dynamic regulation of membrane association via cycles of palmitoylation/depalmitoylation. Palmitoyl acyltransferases (PATs) catalyze the attachment of the palmitoyl group to a cysteine residue in their substrate proteins. We evaluated the molecular evolution of the PAT family in kinetoplastids, and established a nomenclature consistent across species. PAT genes from 27 currently available kinetoplastid genomes were identified and characterized using the PFAM zf-DHHC (PF01529) domain from T. brucei, S. cerevisiae, and H. sapiens as the initial queries for three iterative cycles of best BLAST searches. We sampled widely across the six eukaryotic supergroups and refined the combined dataset using ClustalW neighbor-joining tree models. The phylogenetic analysis was performed using MrBayes with 1 million generations. These comparative genomic and phylogenetic reconstructions documented 18 unique family members in kinetoplastids, of which 6 are pan-eukaryotic orthologues and 12 are unique to this order. A subset of these proteins also displays novel domain architecture. Leishmania species exhibited the most diverse set of PATs, with one genus-specific PAT and additional instances of paralogous expansion. Another intracellular parasite, T. cruzi, had an expanded PAT repertoire, although the free-living Bodo saltans shared similar complexity. In contrast, the T. brucei clade exhibited several instances of secondary loss. These results suggest a complex history of PAT gene expansion and domain fusion in the kinetoplastid ancestor, followed by lineage-specific paralogous duplication or secondary loss.

225 Non-Mitochondrial mRNA Uridylation in T.brucei

***Knusel, Sebastian (Boston University)**; Zhang, Liye (Boston University); Roditi, Isabel (University of Bern); Aphasizhev, Ruslan (Boston University)*

The addition of uridine residues (uridylation or U-tailing) to mRNA 3' ends has been described in several eukaryotes. This unconventional modification is emerging as a universal element triggering transcript degradation. We previously reported U-tailing of a developmentally regulated mRNA (GPEET procyclin) in T. brucei , as well as two non-mitochondrial terminal uridyltransferases (TUTases), TUT3 and TUT4. T. brucei represents a promising model to determine the significance of U-tailing for gene expression, since trypanosomatids rely on post-transcriptional regulation of gene expression. To determine the frequency of adenylated and U-tailed mRNAs on a global scale, we adapted the TAIL-Seq strategy (Chang et al. 2014) to T. brucei . We obtained RNA at various time points during in vitro differentiation of AnTat1.1 bloodstream forms to late procyclic forms, allowing us to investigate whether U-tailing is developmentally regulated. Moreover, TAIL-Seq in mutant parasites should reveal the responsible TUTase and the nuclease(s) degrading U-tailed mRNAs. We also established approaches to identify the RNA substrates and proteins associated with TUTases. Importantly, it is not known whether U-tails in T. brucei decorate mature cytoplasmic mRNAs or their nuclear precursors. We are developing a quantitative RT-PCR approach to discriminate between these two scenarios.

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

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Microbial social behaviors influence life on earth, from nutrient cycling to formation of destructive biofilms. Studies of cell-cell communication and social behavior in bacteria have significantly deepened our understanding of microbial physiology, signaling and pathogenesis. However, mechanisms underlying social behavior in protozoan parasites are mostly unknown. The protozoan parasite, Trypanosoma brucei causes African sleeping sickness, contributing to substantial human suffering and economic hardship in some of the world's most impoverished regions. It is transmitted to a mammalian host through the bite of a tsetse fly vector, where it lives in constant contact with tissue surfaces. When cultivated on surfaces procyclic form (fly midgut stage), T. brucei parasites assemble into multicellular groups that sense and respond to extracellular signals, a behavior we termed social motility. Previous work showed that this social behavior relies on cAMP regulatory systems in the flagellum. Through molecular and systems approaches, we aim to further understand mechanisms of T. brucei social motility. To understand its role in regulating cAMP in the flagellum, we are performing structure-function analyses of adenylate cyclase 1 (AC1) through deletion and site-directed mutagenesis of the AC1 gene. We are also utilizing transcriptomic and proteomic analyses of social motility mutants to identify genes involved in social motility. Additionally, we will identify candidate signals driving social motility through metabolic analysis of the environment surrounding T. brucei engaging in social behavior. This work is still in the beginning stages, but we expect to gain a greater understanding of how T. brucei senses and responds to extracellular signals in the host environment and ultimately, how these processes impact transmission through the fly and mammalian hosts.

227 Elucidating the Mechanism of TLF Mediated Trypanosome Killing Activity

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Humans and certain other primates are immune to infection by Trypanosoma brucei brucei through the activity of Trypanosome Lytic Factor (TLF), which is a subset of High Density Lipoprotein particles. Apolipoprotein L-1 (APOL-1) is the pore forming protein present in TLF that is responsible for killing trypanosomes. The current dogma is that TLF is taken up into the trypanosome via the endocytic pathway and ApoL-1 mediated pore formation occurs after exposure to the acidic environment of the lysosome which results in the influx of water followed by swelling and bursting. Both light microscopic and scanning electron micrograph images have implicated swollen lysosome-like vacuoles as an important part of trypanosome killing by TLF. We aim to first confirm that TLF mediated lysosomal swelling does occur through the use of fluorescent lysosomal and ApoL-1 recognizing antibodies, and then further elucidate what changes occur in the intracellular environment after pore formation through the use of fluorescent pH sensitive indicators.

228 High throughput screening assay to identify novel inhibitors of Trypanosoma brucei from a collection of botanical extracts and small molecules

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Human African Trypanosomiasis, also known as African Sleeping Sickness, is a devastating disease caused by the protozoan parasite, Trypanosoma brucei . The disease is spread to humans by the bite of infected tsetse flies which are endemic to the African continent, where an estimated 70 million people are at risk of contracting the disease. Initial stages of the disease are similar to flu-like symptoms. However, without treatment the disease eventually progresses to debilitating central nervous system involvement and death. Although there are treatments for sleeping sickness, these treatments are very toxic, expensive, and there are growing concerns about efficacy. There is a serious need to find less toxic and more effective treatments for this disease. In this study, 180 botanical extracts from plants used in traditional Chinese medicine and 49 designed small molecules were screened for activity against Trypanosoma brucei . Thirty-three extracts that showed high anti-trypanosomal activity and low cytotoxicity are being further fractionated and studied to identify active compounds that may be useful in the development of new drug therapies for this disease. Three of the small molecules produced IC50 values <15 uM against T. brucei with minimal toxicity.

229 Cis and Trans Effects of a Regulatory RNA Helicase on Substrate Loading and Maturation in the RNA Editing Apparatus in Kinetoplastids.

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The kinetoplastidae diverged approximately 800 million years ago from other eukaryotic lineages. Among other unique biological processes, these protozoa exhibit unprecedented RNA editing by U-insertion/deletion in the mitochondrial mRNA transcriptome. This editing in T. brucei is massive and involves hundreds of small guide RNAs (gRNAs). Notably, studies over 15 years ago established that the central catalytic RNA editing core complex (RECC) does not contain mRNA or gRNA. However, how the RECC enzyme accesses the editing substrates has been a long-standing question. Several accessory editing factors have emerged in recent years including a large and dynamic mitochondrial RNA binding complex 1 (MRB1) that binds gRNA, exhibits weak interactions with RECC) and controls editing by unknown mechanisms. We found that native MRB1 complexes carry pre-mRNA substrates and fully-edited mRNAs [RNA. 2014 Jul;20(7):1142-52]. Because, these complexes contain mRNA and gRNA, and transiently interacts with the RECC enzyme , we proposed that the hybrid substrates are assembled and processed directly on MRB1 scaffolds. We also identified MRB1 variants that differ in protein and gRNA composition, and exhibit specialized editing functions (PLOS ONE, under revision). We traced these functional differences to two discrete RNPs: The gRNA-Associated Protein Complex (GAPC), which is a particularly active form of MRB1 in RNA editing, and the RNA-helicase 2 Complex (REH2C) that contains a critical RNA helicase (REH2) with RNA-binding cofactors. Our focus in this study is on trans effects that we identified of REH2 on the loading of pre-mRNA substrates and their editing in GAPC. We also describe cis effects of conserved domains of REH2 on the RNA-dependent association of this helicase with MRB1 proteins. Our current studies of REH2C apply homology modeling of the helicase and novel RNA-Seq of the edited transcriptome to address the complexities of this massive process. These results are the basis of our updated model of MRB1 function and organization.

230 Substrate-bound RNA helicase and Scaffold RNPs Control RNA Editing in kinetoplastids

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Trypanosoma brucei requires a unique RNA editing by U/insertion-deletion in mitochondrial mRNAs. Editing is directed by a myriad of small guide RNAs (gRNAs) and controlled by unknown mechanisms. This editing modifies over 3,000 sites in mRNAs, and could be controlled during the recruitment of substrates and enzyme, or at initiation and progression, since trypanosomes do not seem to employ transcriptional regulation. A key player in editing is a large and dynamic mitochondrial RNA binding complex 1 (MRB1) that binds gRNA, exhibits weak interactions with the catalytic RNA editing core complex (RECC) and has several proteins that affect editing. Despite much progress in the last few years, it is still unknown how the RECC enzyme is controlled. We reported that native MRB1 complexes carry mRNAs involved in editing [RNA. 2014 Jul;20(7):1142-52]. Thus, MRB1 contains mRNA and gRNA, and transiently interacts with the RECC enzyme. Because RECC does not stably bind RNA, we proposed that editing occurs directly on MRB1. We recently discovered that MRB1 includes two RNPs with specialized functions: the gRNA-Associated Protein Complex (GAPC), that serves as a scaffold for the assembly of editing substrates and processing by RECC; and the RNA Editing Helicase 2 Complex (REH2C) that contains a regulatory RNA helicase and specific helicase cofactors. Here we focus on the REH2 cofactors, which are RNA-binding proteins, and their effects on the functional interplay between REH2C, GAPC and RECC. These editing complexes are recruited in the editing holoenzyme, also known as the editosome. This work and our new preliminary findings establish a new paradigm in MRB1 function and organization that opens new paths to systematically study the regulation of RNA editing. Since this RNA editing is unique to kinetoplastid protozoa, its basic steps and regulation provide attractive targets for therapeutic intervention against parasites of great medical importance.

231 TWO RELATED TRYPANOSOMATID eIF4G HOMOLOGUES HAVE FUNCTIONAL DIFFERENCES COMPATIBLE WITH DISTINCT ROLES DURING TRANSLATION INITIATION

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In higher eukaryotes, eIF4A, eIF4E and eIF4G form the eIF4F complex that facilitates ribosome recruitment to the mRNA and thus the initiation of translation. eIF4G acts as a scaffold for the complex and also interacts with other proteins of the translational machinery. Trypanosomatid protozoa have multiple homologues of eIF4E and eIF4G and the precise function of each remains unclear. Here, two eIF4G homologues, EIF4G3 and EIF4G4, were further investigated. In vitro , both homologues bound EIF4AI, but with different apparent affinities. Binding to distinct eIF4Es was also confirmed; EIF4G3 bound EIF4E4 and EIF4G4 bound EIF4E3, both these interactions required similar binding motifs. EIF4G3, but not EIF4G4, interacted with PABP1, a poly-A binding protein homolog. Work in vivo with Trypanosoma brucei showed that both EIF4G3 and EIF4G4 are cytoplasmic and essential for viability. Depletion of EIF4G3 caused a rapid reduction in global translation whilst EIF4G4 depletion led to growth arrest and changes in morphology but no substantial inhibition of translation. Site-directed mutagenesis was used to disrupt interactions of the eIF4Gs with either eIF4E or eIF4A, causing different levels of growth inhibition. These results are consistent with two distinct eIF4F-like complexes in trypanosomatids but overall only EIF4G3 , with its cap binding partner EIF4E4, may play a major role in translation initiation.

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