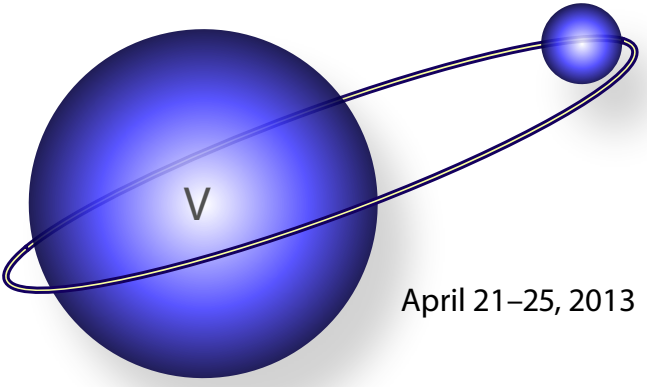
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory · Woods Hole · MA · USA



April 21–25, 2013

KMCBM 2013 Acknowledgements

Fifth Kinetoplastid Molecular Cell Biology Meeting, April 21 – 25, 2013

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)
David Horn (LSHTM, London, UK)
Torsten Ochsenreiter (University of Bern, Bern, Switzerland)
Marc Ouellette (Université Laval, Québec, Canada)

The Staff at MBL:

Elisabeth McCarthy, and all of the Front Desk and Housing staff for registration and housing; Chris Dematos for website design 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 2013 Program Schedule

Sunday, April 21

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

Monday, April 22

07:00 – 08:30	Breakfast
08:45 – 11:45	Session II: Nucleus and Kinetoplast (chair: Sergio Schenkman)
12:00 – 01:30	Lunch
02:00 – 04:30	Session III: Drugs and Methods (chair: Michael Pollastri)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session A
09:00 – 11:00	Mixer & Poster A/B Changeover

Tuesday, April 23

07:00 – 08:30	Breakfast
08:45 – 11:45	Session IV: RNA Metabolism (chair: Keith Matthews)
12:00 – 01:30	Lunch
02:00 – 05:15	Panel Discussions
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session B

09:00 – 11:00	Mixer & Poster B/C Changeover

Wednesday, April 24

07:00 – 08:30	Breakfast
08:45 – 11:45	Session V: Biochemistry (chair: Nicola Carter)
12:00 – 01:30	Lunch
02:00 – 04:45	Session VI: Cell Biology II (chair: Luisa Figueiredo)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session C
09:00 – 11:00	Mixer

Thursday, April 25

07:00 – 08:30	Breakfast
08:45 – 11:15	Session VII: Gene Expression (chair: George Cross)
12:00 – 01:30	Lunch or lunch boxes

ROOMS MUST BE VACATED BY 10:00 AM

ON THURSDAY

Talk Session I: Cell Biology I
(Chair) Kent Hill

04/21/2013

1A Genome wide dissection of the quorum sensing signaling pathway in Trypanosoma brucei
***M. Mony, Binny**; MacGregor, Paula; Cowton, Andrew; Ivens, Alasdair; Horn, David; R. Matthews, Keith*

1B An RNAi library screen identifies proteins that sensitize African trypanosomes to elevated cAMP, suggesting a novel signaling pathway
***Bachmaier, Sabine**; Gould, Matthew K.; Ali, Juma A. M.; Munday, Jane; Horn, David; de Koning, Harry P.; Boshart, Michael*

1C Role of the Trypanosoma brucei BBSome in host-pathogen interaction
***Langousis, Gerasimos**; Hill, Kent L.; Shimogawa, Michelle; Edwin, Saada; Ng, Eva*

1D The global view: Characterisation of organelle segregation and distribution throughout the T. brucei bloodstream form cell cycle.
***Vaughan, Sue**; Hughes, Louise; Towers, Katie; Barry, Samantha; Starborg, Tobias; Gull, Keith*

1E Identification of kinetochore proteins in Trypanosoma brucei *Akiyoshi, Bungo; Gull, Keith*

1F Kharon1 mediates flagellar targeting of a glucose transporter in Leishmania mexicana
***Tran, Khoa D.**; Rodriguez-Contreras, Dayana; Vieira, Danielle; Landfear, Scott M.*

1G A specific life-cycle stage of Trypanosoma brucei exhibits social motility *Imhof, Simon; Roditi, Isabel*

1H Paratrypanosoma – the earliest branch of trypanosomatids and the changing biodiversity landscape of the monoxenous parasites
***Lukes, Julius**; Votypka, Jan; Flegontov, Pavel; Skalicky, Tomas; Tyc, Jiri; Jirku, Milan; Yurchenko, Vyacheslav; Archibald, John M.; Maslov, Dmitri A.*

Talk Session II: Nucleus and Kinetoplast

(Chair) Sergio Schenkman

04/22/2013

2A Genome-wide organisation of DNA replication initiation in kinetoplastid parasites
***McCulloch, Richard**; Dickens, Nicholas; Marques, Catarina; Marcello, Lucio*

2B An in vitro nucleosome reconstitution system to study chromatin modification by trypanosome DOT1 methyltransferases
***Dindar, Guelcin**; Mehlhorn, Christine; Schneider, Ludmila; Anger, Andreas; Hake, Sandra; Janzen, Christian*

2C Uncovering evolution in 3D: Architecture of the trypanosome nuclear pore complex reveals conserved and divergent features across a billion years
***Obado, Samson**; Brilliantes, Marc; Zhang, Wenzhu; Field, Mark; Chait, Brian; Rout, Michael*

2D TbNMD3 regulates mRNA via an Xpol-linked nuclear export mechanism. ***Walrad, Pegine**; Buehlmann, Melanie; Capewell, Paul; Arunasalam, Naguleswaran; Roditi, Isabel; Ullu, Elisabetta; Matthews, Keith R.*

Coffee Break

2E The telomere DNA binding activity of Trypanosoma brucei TRF is important for suppression of sub-telomeric VSG switching
***Benmerzouga, Imaan**; Li, Xiaohua; Yanxiang, Zhao; Li, Bibo*

2F Antigenic variation in T. brucei: evidence for VSG allelic exclusion through sequence-specific genetic interference
***Hutchinson, Sebastian**; Alsford, Sam; Horn, David*

2G Base J Insertion and Function in Leishmania
***Baugh, Loren**; Borst, Piet; Genest, Paul-Andre; Myler, Peter J.; van Luenen, Henri*

2H A trans-spliced telomerase RNA dictates telomere synthesis in Trypanosoma brucei
***Li, Bibo**; Sandhu, Ranjodh; Sanford, Samantha; Basu, Shrabani; Park, MinA; Pandya, Unnati; Chakrabarti, Kausik*

2I Control of Trypanosoma brucei kDNA replication by the TbHSLVU Protease
***Tiengwe, Calvin**; Englund, Paul T; Jensen, Robert E*

2J A conserved mitochondrial outer membrane protein mediates kDNA maintenace in Trypanosoma brucei
***Schnarwiler, Felix**; Dewar, Caroline; Schnaufer, Achim; Schneider, André*

Talk Session III: Drugs and Methods

(Chair) Michael Pollastri

04/22/2013

3A Vacuolar ATPase knockdown in T. brucei allows for kinetoplast-independent growth and resistance to the major veterinary drug isometamidium
***Baker, Nicola**; Barrett, Michael P.; Horn, David*

3B Identification of kinetoplastid deubiquitinating enzymes and their inhibitors reveal a novel target-lead pair for antiparasite drug development
***Rodenko, Boris**; Yalçin, Zeliha; Monnier, Annelie; Moss, Catherine; Brown, Elaine; Celie, Patrick; Burchmore, Richard; Ovaa, Huib; Mottram, Jeremy*

3C New progress on the mode of action of and tolerance to miltefosine in L. infantum using metabolomics
***Vincent, Isabel M.**; Weidt, Stefan; Rivas, Luis; Burgess, Karl; Ouellette, Marc*

3D Inhibition of Trypanosoma brucei cathepsin-L increases sensitivity to lysis by human serum
***Alsford, Sam**; Horn, David*

Coffee Break

3E The dynamic nature of experimental chronic Chagas disease revealed by highly sensitive in vivo imaging
***Lewis, Michael**; Burrell-Saward, Hollie; Fortes Francisco, Amanda; Kelly, John; McLatchie, Alex; Miles, Michael; Taylor, Martin*

3F Expression GPIomics of the Mammal-Dwelling Stages of Trypanosoma cruzi
***Lopes, Felipe G.**; Aguilar, Clemente; C. Almeida, Igor; F. Marques, Alexandre; S. Nakayasu, Ernesto*

3G Quantitative phosphoproteomic analysis of procyclic and bloodstream form Trypanosoma brucei reveals potential roles for phosphorylation in adaptation
***Urbaniak, Michael**; Ferguson, Michael A.J.; Guther, M. Luca S.; Martin, David M. A.*

3H Use of the HaloTag to modulate protein level in Trypanosoma cruzi and Trypanosoma brucei.
***Jones, Deuan**; De Rycker, Manu; Fairlamb, Alan; Patterson, Stephen*

3I Identification of the mRNA-fate modulators through a high-throughput tethering assay
***Erben, Esteban D.**; Clayton, Christine; Fadda, Abeer; H.V. e Vieira, Carlos*

Talk Session IV: RNA Metabolism

(Chair) Keith Matthews

04/23/2013

4A Interactions of LEISHMANIA RNA Virus 1 (LRV1) with the RNA interference pathway

***Beverley, Stephen M.**; Lye, Lon-Fye; Akopyants, Natalia; Shaik, Jahangheer; Brettman, Erin; Kuhlmann, F. Matthew; Zangger, Haroun; Ronet, Catherine; Fasel, Nicolas*

4B Leishmania aethiopica field isolates with an immunogenic Leishmania RNA virus

***Zangger, Haroun**; Desponds, Chantal; Lye, Lon-Fye; Hailu, Asrat; Beverley, Stephen; Fasel, Nicolas*

4C The Trypanosoma brucei siRNA exonuclease TbRIF4 has a second role in the maintenance of TbAGO1 protein levels

***Barnes, Rebecca**; Ramey-Butler, Kiantra; Kolev, Nikolay; Shi, Huafang; Tschudi, Christian; Ullu, Elisabetta*

4D Regulation of RNA metabolism by ARE-binding proteins in Trypanosoma brucei

***Lu, Zhiquan**; Najafabadi, Hamed Shateri; Mehta, Vaibhav; Gazestani, Vahid Hajihoseini; Adoue, Veronique; Salavati, Reza*

Coffee Break

4E Genome-wide RNA-binding analysis of the trypanosome U1 snRNP-specific proteins U1C and U1-70K provides evidence for a spliceosomal network linking cis- and trans-splicing

***Preußer, Christian**; Roßbach, Oliver; Hung, Lee-Hsueh; Li, Dan; Bindereif, Albrecht*

4F Architecture of the RNA Editing Holoenzyme

***Afasizhev, Ruslan**; Huang, Lan; Afasizheva, Inna*

4G Mechanism of spliced leader RNA silencing (SLS) and factors involved in the controlling the of SLS-induced programmed cell death

***Michaeli, Shulamit**; Hope, Ronen; Ben-Mayor, Efrat; Biswas, Dipul; Voloshin, Konstatin; Gunzl, Arthur*

4H Genome-wide analysis of translation in Trypanosoma brucei using ribosome profiling

***Jensen, Bryan C.**; Ramasamy, Gowthaman; Haydock, Andrew; Ingolia, Nicholas; Myler, Peter; Parsons, Marilyn*

4I High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome

***hashem, yaser**; Des Georges, Amedee; Fu, Jie; Buss, Sarah N.; Jossinet, Fabrice; Jobe, Amy; Zhang, Qin; Liao, Hstau Y.; Grassucci, Robert A.; Bajaj, Chandrajit; Westhof, Eric; Madison-antenucci, Susan; Frank, Joachim*

Talk Session V: Biochemistry
(Chair) Nicola Carter

04/24/2013

5A Lipid metabolism in Trypanosoma brucei and other kinetoplastids: so much more than just membranes!
***SMITH, TERRY K.**; YOUNG, SIMON; MAJOR, LOUISE; DENTON, HELEN*

5B Leishmania Rely on Ethanolamine for Phospholipid Synthesis
***Pawlowic, Mattie C.**; Hsu, Fong-Fu; Zhang, Kai*

5C Trypanosomatid deoxyhypusine synthase requires heterotetramer formation with a catalytically dead paralog which serves as an activator domain.
***Nguyen, Suong**; Jones, Deuan; Wyllie, Susan; Fairlamb, Alan; Phillips, Margaret*

5D Intracellular amastigote stages of Leishmania mexicana exhibit a limited capacity to utilize carbon sources other than sugars.
***Saunders, Eleanor**; Ng, William; Kloehn, Joachim; Chambers, Jennifer; Ng, Milica; McConville, Malcolm*

Coffee Break

5E Cytosolic NADPH homeostasis in glucose-starved procyclic Trypanosoma brucei relies on malic enzyme and gluconeogenic flux
***Allmann, Stefan**; Morand, Pauline; Ebikeme, Charles; Gales, Lara; Michels, Paul; Portais, Jean-Charles; Boshart, Michael; Bringaud, Frédéric*

5F A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in *Leishmania donovani*.
***Martin, Jessica L.**; Boitz, Jan; Carter, Nicola; Cassera, Maria Belan; Fulwiler, Audrey; Ullman, Buddy; Yates, Phillip*

5G GMPS: The "Achilles's Heel" of Purine Metabolism in *Leishmania donovani*
***Boitz, Jan M.**; Fulwiler, Audrey; Ullman, Buddy*

5H The ATOM complex: gate to the trypanosome mitochondrion
***Mani, Jan**; Pusnik, Mascha; Niemann, Moritz; Schmidt, Oliver; Oeljeklaus, Silke; Wiese, Sebastian; Warscheid, Bettina; Meisinger, Chris; Schneider, André*

5I The essential Leishmania major MAP kinase LmaMPK4 is involved in pH sensing and differentiation of metacyclic promastigotes
***Dacher, Mariko**; Beverley, Stephen M.; Leclercq, Olivier; Morales, Miguel A.; Pescher, Paschale; Spath, Gerald F.*

5J The Leishmania MAP kinase MPK10 is essential for L. donovani axenic amastigote development and regulated by a parasite-specific auto-inhibitory domain.
***Cayla, Mathieu**; Rachidi, Najma; Schmidt-Arras, Dirk; Rosenqvist, Heidi; Jensen, Ole Nørregaard; Wiese, Martin; Späth, Gerald*

Talk Session VI: Cell Biology II
(Chair) Luisa Figueiredo

04/24/2013

6A How to evade capture: VSG trafficking and maintaining the African trypanosome surface

***Field, Mark F.**; Manna, Paul; Boehm, Cordula; Gadelha, Catarina; Adung'a, Vincent; Obado, Samson; Chait, Brian T.; Rout, Michael P.*

6B Analysis of genetic exchange in experimental crosses of Leishmania major using Next Generation Sequencing Data

***Shaik, Jahangheer S.**; Akopyants, Natalia; Dobson, Deborah; Lawyer, Phillip; Elnaiem, Dia-Eldin; Sacks, David; Beverley, Stephen*

6C Three dimensional organisation of connections between basal bodies and the kinetoplast.

***Towers, Katie**; Hughes, Louise; Gull, Keith; Vaughan, Sue*

Coffee Break

6D A novel component of the flagellum attachment zone (FAZ) and its role in FAZ assembly, cytokinesis, and cell morphogenesis in Trypanosoma brucei

***Zhou, Qing**; Li, Ziyin*

6E IRON UPTAKE IN TRYPANOSOMA BRUCEI

***Taylor, Martin C.**; McLatchie, Alex; Thomas, James; Ntais, Dionysios; Kelly, John*

6F Iron regulated modulation in ROS levels: a novel signaling mechanism for Leishmania differentiation.

***Mittra, Bidyottam**; Menezes, Juliana; Miguel, Danilo; Andrews, Norma*

6G Detecting and defining subdomains at the surface of African trypanosomes by proteomics

***Gadelha, Catarina**; Chait, Brian T.; Field, Mark C.; Zhang, Wenzhu*

6H BioID for trypanosomes - novel bilobe components identified by proximity to TbMORN1

Morriswood, Brooke; *Havlicek, Katharina; Demmel, Lars; Yavuz, Sevil; Sealey-Cardona, Marco; Vidilaseris, Keni; Kostan, Julius; Djinovic-Carugo, Kristina; Roux, Kyle; Warren, Graham*

Talk Session VII: Gene Expression

(Chair) George Cross

04/25/2013

7A Characterization of cdc2-related kinase 9 (CRK9), a key enzyme in trypanosome gene expression
***Badjatia, Nitika**; Ambrosio, Daniela; Nguyen, Bao; Günzl, Arthur*

7B Characterization of the Small Proteome of Trypanosoma brucei
***Ericson, Megan**; Janes, Michael; Butter, Falk; Obado, Samson; Rout, Michael; Mann, Matthias; Ullu, Elisabetta; Tschudi, Christian*

7C VEX1 controls VSG allelic exclusion and antigenic variation in T. brucei
***Glover, Lucy K.**; Horn, David*

7D Chromatin SUMOylation by the SUMO E3 ligase SIZ1 Promotes VSG Expression Site Transcription in T. brucei.
***López-Farfán, Diana**; Bart, Jean-Mathieu; Navarro, Miguel*

7E Chromatin readers regulate monoallelic expression and switching in T. brucei
***Schulz, Danae**; Papavasiliou, Nina*

7F Histone H1: a linker between chromatin, gene expression and parasite virulence in Trypanosoma brucei
***Pena, Ana C.**; Pimentel, Mafalda R.; Manso, Helena; Ferreira, Filipa R.; Neves, Daniel; Aresta-Branco, Francisco; Guegan, Fabien; Coelho, Luis P.; Figueiredo, Luisa M.*

7G The multiple cap-binding protein paralogs enable Leishmania to survive a multitude of physiological stresses
***Shapira, Michal**; Zinoviev, Alexandra; Leger, Melissa; Wagner, Gerhard*

7H A DEAD box RNA helicase homolog plays a key role in Leishmania amastigote differentiation and adaptation to intracellular stress
***Samant, Mukesh**; Padmanabhan, Prasad; Dumas, Carole; Dupé, Aurélien; Papadopoulou, Barbara*

7I The role of RNA-binding protein RBP6 in mediating progression through the insect life-cycle stages of Trypanosoma brucei
***Kolev, Nikolay G.**; Ramey-Butler, Kiantra; Shi, Huafang; Janes, Michael; Ericson, Megan; Tomaino, Francesca; Ullu, Elisabetta; Tschudi, Christian*

Poster Session: A

04/22/2013

77 Acidocalcisome-mediated autophagic regulation in Trypanosoma brucei
***Li, Fengjun**; He, Cynthia Yingxin*

78 Role of Centrins in Cytokinesis and Organelle Mis-segregation in Trypanosoma brucei
***Selvapandiyan, Angamuthu**; Kumar, Praveen; Salisbury, Jeffrey; Wang, Ching; Nakhasi, Hira*

79 Eighteen amino acids at the N terminal tip of Leishmania proline/alanine transporter determine substrate specificity
***Schlisselberg, Doreen**; Inbar, Ehud; Rentsch, Doris; Myler, Peter; Zilberstein, Dan*

80 Characterization of the glycerol-3-phosphate acyltransferase TbGAT of Trypanosoma brucei
***Zufferey, Rachel**; Patel, Nipul; Pirani, Karim; Dahlstrom, Kelly; Zhu, Tongtong; Dhalladoo, Subbhalakshmi; Chaitan, Veronica; Cheung-See-Kit, Melanie*

81 The role of the flagellum attachment zone in subpellicular microtubule organization
***Sheriff, Omar**; He, Cynthia*

82 The trypanosomal pumilio protein TbPUF2 is involved in the regulation of mRNAs coding for cytoskeletal proteins
***Jha, Bhaskar Anand**; Fadda, Abeer; Clayton, Christine*

83 Quantitative phosphoproteomic analysis of procyclic and bloodstream form Trypanosoma brucei reveals potential roles for phosphorylation in adaptation
***Urbaniak, Michael D.**; Guther, M. Lucia S.; Martin, David M. A.; Ferguson, Michael A. J.*

84 Trypanothione-dependent peroxidases protect African trypanosomes from lysosomal oxidative stress.
***Hiller, Corinna**; Krauth-Siegel, R. Luise*

85 Purification of specific mRNPs via the nascent peptide
***Inchaustegui Gil, Diana**; Clayton, Christine*

86 Sterol biosynthesis in Leishmania
***Xu, Wei**; Hsu, Fong-Fu; Zhang, Kai*

87 Translational activator complex in mitochondria of Trypanosoma brucei
***Maslov, Dmitri A.**; Ridlon, Lucie; Skodova, Ingrid; Lukes, Julius*

88 The flagellar pocket collar protein 4 (FPC4): A BILBO1-microtubule linker?
***ROBINSON, Derrick R.**; FLORIMOND, Célia; EGGENSPIELER, Marie; SAHIN, Annelise; DACHEUX, Denis; LANDREIN, Nicolas; BONHIVERS, Melanie*

89 Insights into the nuclear mRNA export machinery of Trypanosoma brucei
***Schimanski, Bernd**; Dostalova, Anna; Käser, Sandro; Cristodero, Marina*

90 Genome-wide mapping of histone H3K4 trimethylation and gene expression regulation in Leishmania
***Gazanion, Elodie**; Joly-Beauparlant, Charles; Droit, Arnaud; Papadopoulou, Barbara; Corbeil, Jacques; Ouellette, Marc*

91 Inhibition of nucleotide sugar transport in Trypanosoma brucei alters surface glycosylation
***Liu, Li**; Xu, Yu-Xin; Caradonna, Kacey; Kruzel, Emilia; Burleigh, Barbara; Bangs, James; Hirschberg, Carlos*

92 Clu(eless) proteins in *Trypanosoma brucei* – Their enigmatic function
***Benz, Corinna**; Lukeš, Julius*

93 MRE11 involvement in DNA repair and drug resistance in Leishmania
***Laffitte, Marie-Claude**; Mukherjee, Angana; Légaré, Danielle; Ouellette, Marc*

94 Para Tryp Research Organization
Lyda, Todd A.

95 Prospects and challenges for repositioning drugs and drug candidates against kinetoplastid diseases
***Campbell, Robert K.**; Pollastri, Michael P.*

96 A specific life-cycle stage of Trypanosoma brucei exhibits social motility
***Imhof, Simon**; Roditi, Isabel*

97 Identification of the mRNA-fate modulators through a high-throughput tethering assay
***Erben, Esteban D.**; Fadda, Abeer; H. V. e Vieira, Carlos; Clayton, Christine*

98 Initiation of nuclear DNA replication in Trypanosoma brucei: a single factor or a complex?
***Marques, Catarina A.**; Dickens, Nicolas J.; Tiengwe, Calvin; Marcello, Lucio; McCulloch, Richard*

99 An approach to determine the Transcriptome of T. b. rhodesiense from Sleeping Sickness Patients in Uganda
***Mulindwa, Julius**; Fadda, Abeer; Matovu, Enock; Enyaru, John; Clayton, Christine*

100 Inositol metabolism in *Trypanosoma brucei*
***Major, Louise L.**; Denton, Helen; Smith, Terry K*

101 Mechanistic insights of SIDER2 retroposon-mediated mRNA decay in Leishmania
***Azizi, Hiva**; Patrícia Romão Pompílio de Melo, Tatiany; Dumas, Carole; Papadopoulou, Barbara*

102 Life without a diamine transporter: a *T. cruzi* perspective
***Hasne, Marie-Pierre**; Ullman, Buddy*

103 Mouse bloodstream infection and pathogenesis is unaffected by disruption of Trypanosome propulsive motility.
***Kisalu, Neville**; Hill, Kent L.; Langousis, Gerasimos; Bentolina, Laurent; Ralston, Katherine; Weiss, Shimon*

104 Expression GPIomics of the Mammal-Dwelling Stages of Trypanosoma cruzi
***Lopes, Felipe G.**; S. Nakayasu, Ernesto; F. Marques, Alexandre; Aguilar, Clemente; C. Almeida, Igor*

105 Involvement of a mitochondrial carrier in drug resistance in T. brucei
***Pereira de Macêdo, Juan**; Schumann Burkard, Gabriela; Roditi, Isabel; Vial, Henri; Bütikofer, Peter*

106 Mismatch repair in Trypanosoma brucei roles in protection against oxidative stress
***Zeb, Tehseen F.**; McCulloch, Richard*

107 Characterization, expression and proteolytic activity of Trypanosoma rangeli Major Surface Proteases (MSPs)
***Pedrosa, Andre L.**; Ferreira, Keila; Calixto, Paulo; Lages-Silva, Eliane; Ramirez, Luis*

108 17-AAG INDUCES INCIDENTAL CELL DEATH OF Leishmania amazonensis WITH AUTOPHAGIC FEATURES
***Petersen, Antonio Luis**; Guedes, Carlos Eduardo; Versoza, Varolina Leite; Cull, Benjamin; Lima, José Geraldo Bomfim; Freitas, Luiz Antônio; Borges, Valeria Matos; Mottram, Jeremy C; Veras, Patricia Sampaio T*

109 The family M17 Leucyl aminopeptidases from the TriTryps
***Timm, Jennifer**; Garcia-Nafria, Javier; Gonzales Pacanowska, Dolores; Wilson, Keith*

110 A model system for investigating VSG switching and coat formation
***Jones, Nicola G.**; Batram, Christopher; Carrington, Mark; Engstler, Markus*

111 Receptor-cargo complexes form in the cytosol of Leishmania donovani for trafficking to the glycosome.
***Strasser, Rona**; Jardim, Armando*

112 Initiation of VSG switching in Trypanosoma brucei
***Devlin, Rebecca**; McCulloch, Richard*

113 Trypanosoma cruzi RNA Polymerase II is phosphorylated during transcription elongation
***Moretti, Nilmar S.**; Rocha, Antônio; Schenkman, Sergio*

114 Mitochondrial outer membrane proteome of T. brucei reveals novel factors required to maintain mitochondrial morphology
***Niemann, Moritz**; Wiese, Sebastian; Mani, Jan; Chanfon, Astrid; Jackson, Christopher; Meisinger, Chris; Warscheid, Bettina; Schneider, André*

115 Chemistry based approach for the identification of a Ufm1 specific processing protease in Leishmania
***Elakhal Naouar, Ines**; Strader, Michael Brad; Duncan, Robert; Nakhasi, Hira L.; Gannavaram, Sreenivas*

116 Evaluation of an aptamer-based assay to detect biomarkers of Trypanosoma cruzi infection in mice treated with Benznidazole
***Fortes de Araujo, Fernanda**; Nagarkatti, Rana; Gupta, Charu; Marino, Ana Paula; Debrabant, Alain*

117 Identification of biomarkers of Trypanosoma cruzi infection in blood using aptamers generated against TESA
***Gupta, Charu**; Nagarkatti, Rana; Mindaye, Samuel; Fortes de Araujo, Fernanda; Debrabant, Alain*

118 Polo-like kinase phosphorylation of bilobe-resident TbCentrin2 facilitates flagellar positioning in Trypanosoma brucei
***de Graffenried, Chris**; Anrather, Dorothea; Von Raußendorf, Freia; Warren, Graham*

119 Insights into the architecture and protein interaction network of RNA editing associated complexes in Trypanosoma brucei
***Nikpour, Najmeh**; Mak, Ivy; Shateri Najafabadi, Hamed; Moshiri, Houtan; Hajihosseini Gazestani, Vahid; Salavati, Reza*

120 Negative feedback control mediated by the 3' untranslated region assuring the low expression level of the RNA binding protein TcRBP19 in T. cruzi epimastigotes
***Pérez Díaz, Leticia**; Pastro, Lucía; Smircich, Pablo; Dallagiovanna, Bruno; Garat, Beatriz*

121 The N-terminal targeting signal of Trypanosome Alternative Oxidase is dispensable for its import into mitochondria
***Hamilton, VaNae N.**; Singha, Ujjal; Weems, Ebony; Chaudhuri, Minu*

122 The Unique Leishmania EIF4E4 N-Terminus is a Target for Multiple Phosphorylation Events and Takes Part in Critical Interactions Required for Translation Initiation
***de Melo Neto, Osvaldo P.**; da Costa Lima, Tamara; Pereira, Mariana; Romao, Tatiany; Papadopoulou, Barbara*

123 Base J Insertion and Function in Leishmania
***Baugh, Loren**; van Luenen, Henri; Genest, Paul-Andre; Borst, Piet; Myler, Peter J.*

124 Comparative transcriptomics of Trypanosoma cruzi primary and secondary amastigogenesis
***Kessler, Rafael L.**; Krieger, Marco Aurelio; Probst, Christian*

125 Spatiotemporal Localization of Mitochondrial DNA Polymerases in Trypanosoma brucei.

***Greene, Rebecca**; Concepcion-Acevedo, Jeniffer; Luo, Juemin; Klingbeil, Michele*

126 Overexpression of a Trypanosoma brucei RNA-Binding Protein in vitro Promotes Metacyclogenesis

***Ramey-Butler, Kiantra I.**; Kolev, Nikolay G.; Shi, Huafang; Janes, Michael; Ullu, Elisabetta; Tschudi, Christian*

127 Haptoglobin-hemoglobin receptor-independent uptake of trypanosome lytic factor 2

***Zipkin, Ron**; Molina-Portela, Pilar; Raper, Jayne*

128 Control of mitochondrial shape in Trypanosoma brucei by TbLOK1, a novel outer membrane protein

***Povelones, Megan L.**; Tiengwe, Calvin; Gluenz, Eva; Gull, Keith; Englund, Paul; Jensen, Robert*

129 A Novel Method for Mitochondrial RNA Knockdown of ATPase Subunit A6 mRNA in Trypanosoma brucei

***Szempruch, Anthony**; Choudhury, Rajarshi; Wang, Zefeng; Hajduk, Stephen*

130 Validating Trypanosoma cruzi spermidine synthase as a target for rational drug design against Chagas disease: an integrated structural biology and molecular genetics approach.

***Gretes, Michael**; Karplus, P Andrew; Hasne, Marie-Pierre; Ullman, Buddy; Hol, Wim G; Bosch, Jürgen*

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132 Evaluation of the Apoptotic-like Activity of Ruthenium-Clotrimazole Compounds against Leishmania major
Iniguez, Eva

133 Identification of transmission stage-specific gene regulators in Trypanosoma brucei via a genome-wide RNAi selection approach
***Rico Vidal, Eva**; Monk, Stephanie; Glover, Lucy; Horn, David; Matthews, Keith*

134 A Single Amino Acid Substitution in the Trypanosoma brucei gambiense Haptoglobin-Hemoglobin Receptor Abolishes TLF-1 Binding
DeJesus, Eric; Hajduk, Stephen

135 Trypanosoma brucei Vacuolar Transporter Chaperone 4 (TbVTC4) is an acidocalcisomal polyphosphate kinase required for in vivo infection
***Lander, Noelia**; Ulrich, Paul; Docampo, Roberto*

136 A mitogen-activated protein kinase is required for basal body duplication and segregation and cytokinesis in Trypanosoma brucei
***Wei, Ying**; Li, Ziyin*

137 Trypanosomes possess a distinct XPB helicase for nucleotide excision repair that functions independently of a TFIIH complex
***Badjatia, Nitika**; Nguyen, Tu; Lee, Ju Huck; Günzl, Arthur*

138 Regulation of mitochondrial function and virulence by a scaffold protein in Leishmania
***Cardenas, Daviel**; Kelly, Ben*

139 ZC3H32 is an essential zinc finger protein that can destabilise RNA
***Klein, Cornelia A.**; Erben, Esteban; Minia, Igor; Singh, Aditi; Clayton, Christine*

140 Trypanosoma brucei contains two Asf1 forms with distinct cellular localization and histone chaperone function
***Schenkman, Sergio**; Pascoalino, Bruno*

141 Characterization of the trypanosome PRP19 complex involved in cis and trans splicing of nuclear pre-mRNA
***Ambrosio, Daniela**; Günzl, Arthur*

142 Mitochondrial production of acetate is essential for viability of the Trypanosoma brucei bloodstream forms
***mazet, muriel**; Morand, pauline; Biran, Marc; bringaud, frédéric; Morand, Patrick; Bouyssou, Guillaume*

143 Pharmacological assessment of the Leishmania casein kinase protein family reveals CK1.2 as important drug target with essential functions in intracellular parasite survival

***Rachidi, Najma**; Durieu, Emilie; Taly, Jean François; Pescher, Pascale; Aulner, Nathalie; Rouault-Hardoin, eline; Notredame, Cedric; Prina, Eric; Meijer, Laurent; Späth, Gerald*

144 Polyphosphate polymerase in Leishmania parasites

***Kohl, Kid**; Mayer, Andreas; Fasel, Nicolas*

145 Functional characterization of Mucin-Associated Surface Protein (MASP) in the human parasite Trypanosoma cruzi

***Choi, Jung Min**; Fernandes Dupecher, Maria Cecilia; Cai, Qian; Cerqueira, Gustavo; Sheng, Zu-Hang; Andrews, Norma W.; El-Sayed, Najib M.*

146 Functional analysis of the ATP synthase gamma subunit mutation that allows kDNA deletion in Trypanosoma brucei

***Dewar, Caroline**; Gould, Matt; Dean, Sam; Schnaufer, Achim*

147 TbNUP-2, a second component of the trypanosome nucleoskeletal lamina

***Maishman, Luke**; Obado, Samson; Alsford, Sam; Bart, Jean-Mathieu; Navarro, Miguel; Horn, David; Chait, Brian; Rout, Michael; Field, Mark*

148 Regulatory mechanisms of Leishmania aquaglyceroporin AQP1

***Mandal, Goutam**; Mandal, Srotoswati; Sharma, Mansi; Orta, Jose; Papadopoulou, Barbara; Mukhopadhyay, Rita*

149 Localization and Function of Palmitoyl Acyltransferase 7 in Trypanosoma brucei

***Goldston, Amanda**; Emmer, Brian; Olson, Cheryl; Lawler, Kimberly; Epting, Conrad; Engman, David*

150 The rich repertoire of Leishmania major small nucleolar RNA: implication on their function in rRNA modification and processing.

***Eliaz, Dror**; Michaeli, Shulamit; Doniger, Tirza; Tkacz, itai Dov; Gupta, Sachin Kumar; Kolev, Nikolay G.; Unger, Ron; Ullu, Elisabetta*

151 Leishmania infantum chagasi Ecto-Nucleoside Triphosphate Diphosphohydrolase: heterologus expression, biochemical characterization and influence on macrophage infection

***Fietto, Juliana**; Vasconcellos, Raphael; Mariotini-Moura, Christiane; Lacerda, Tonielle; Borges-Pereira, Lucas; Donatelli, Tiago; Gomes, Rodrigo Saar; Baqui, Munira; Almeida, Márcia; Afonso, Luís Carlos; Júnior, Abelardo Silva; Bressan, Gustavo Costa*

152 Functional contribution of Pds5 to sister chromatid cohesion in T. brucei

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

153 Phosphatidylethanolamine synthesis in Trypanosoma brucei

***Farine, Luce**; Bütikofer, Peter*

154 Characterization of a heteromeric potassium channel essential for the bloodstream form of T. brucei

***Steinmann, Michael**; Mäser, Pascal; Bütikofer, Peter; Sigel, Erwin*

155 Using chromatin-based strategies to identify transcription-associated elements in Leishmania major

***Anderson, Britta**; Shaik, Jahangheer; Beverley, Stephen*

156 Characterization of the Late Endosome and ESCRT Machinery in *Trypanosoma brucei*

***Silverman, Jason**; Bangs, Jay*

157 Dual core processing: MRB1 is an essential trypanosome RNA editing complex

***Ammerman, Michelle**; Simpson, Rachel; Hashimi, Hassan; Kafkova, Lucie; Faktorova, Drahomira; Lukes, Julius; Read, Laurie*

158 Trypanosoma brucei TIN2 inhibits VSG switching by maintaining the subtelomere integrity

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

159 A calpain-like protein regulates cell shape in Trypanosoma brucei

***Sunter, Jack D.**; Varga, Vladimir; Hayes, Polly; Olego-Fernandez, Sofia; Ginger, Michael; Gull, Keith*

160 The dynamic nature of experimental chronic Chagas disease revealed by highly sensitive in vivo imaging

***Lewis, Michael**; Fortes Francisco, Amanda; Taylor, Martin; Burrell-Saward, Hollie; McLatchie, Alex; Miles, Michael; Kelly, John*

161 The VSG 3'UTR and regulation of VSG expression

***Trenaman, Anna**; Sherwitzl, Iris; Wand, Nadina; Narayanan, Mani Shankar; Rudenko, Gloria*

162 Interaction of Leishmania donovani PEX14 with glycosomal membrane

***Kottarampatel, Anwer Hasil**; Cyr, Normand; Strasser, Rona; Jardim, Armando*

163 Correlating lifestyle and trafficking systems in kinetoplastids: The evolutionary history of Rab and SNARE proteins

***Venkatesh, Divya**; J O'Reilly, Amanda; T. Manna, Paul; Kelly, Steve; C. Field, Mark*

164 Environmentally regulated localization of Trypanosoma brucei hexokinase 2

***Kahney, Elizabeth W.**; Joice, April; Singha, Ujjal; Chaudhuri, Minu; Morris, James*

165 Investigation of cis-elements involved in regulation of gene expression in Leishmania

***Terrão, Monica C.**; Vasconcelos, Elton J. R.; Ruiz, Jerônimo C.; Vêncio, Ricardo Z.; Ramasamy, Gowthaman; Myler, Peter J.; Cruz, Angela K.*

166 Host fatty acid metabolism and growth of intracellular Trypanosoma cruzi amastigotes

***Shah-Simpson, Sheena**; Caradonna, Kacey; Burleigh, Barbara*

167 Novel Therapeutics for Human and Animal African Trypanosomiasis
***Harrington, John M.**; Friedman, Joel; Hajduk, Stephen*

168 Implication of Leishmania MAPKs in miltefosine resistance
***Vacchina, Paola**; Luque-Ortega, Juan R.; Prina, Eric; Rivas, Luis; Späth, Gerald F.; Morales, Miguel A.*

169 The role of KBP17, a novel Trypanosoma brucei kDNA-binding protein, in kinetoplast DNA replication.
***Allary, Marina**; Acestor, Nathalie; Coppens, Isabelle; Panigrahi, Aswini; Englund, Paul; Jensen, Robert*

170 Leishmania-macrophage interactions: novel mechanisms of antioxidant/antinitrosative defense.
***Henard, Calvin**; Carlson, Eric; Hay, Christie; Soong, Lynn*

171 Cell surface proteomes from insect-form and mammalian bloodstream-form Trypanosoma brucei
***Shimogawa, Michelle**; Vashisht, Ajay; Saada, Edwin; Wohlschlegel, James; Hill, Kent*

172 Fucosylation in Trypanosoma brucei
***Damerow, Sebastian**; Bandini, Giulia; Guo, Hongjie; Beverley, Stephen; Ferguson, Michael*

173 Use of the HaloTag to modulate protein level in Trypanosoma cruzi and Trypanosoma brucei.
***Jones, Deuan**; Patterson, Stephen; De Rycker, Manu; Fairlamb, Alan*

174 Trypomastigotes and amastigotes of Trypanosoma cruzi induce apoptosis and STAT3 activation in cardiomyocytes in vitro
***Stahl, Philipp**; Ruppert, Volker; Meyer, Thomas; Campos, Marco A.; Gazinelli, Ricardo T.; Maisch, Bernhard; Schwarz, Ralph T.; Debierre-Grockiego, Françoise*

175 Stage-specific Differences in the Regulation of T. brucei Acetyl-CoA Carboxylase by Environmental Lipids
***Ray, Sunayan S.**; Paul, Kimberly*

176 An aminoacyl-tRNA synthetase complex is required for efficient tRNA-aminoacylation in African trypanosomes
***Cestari, Igor**; Kalidas, Savitha; Anupama, Atashi; Phillips, Margaret A.; Stuart, Kenneth*

177 Genomic analysis of sequence-dependent DNA curvature in Leishmania
***Garat, Beatriz**; Smircich, Pablo; Forteza, Diego; El-Sayed, Najib*

178 Targeting Protein Kinases in Trypanosoma brucei
***Merritt, Chris**; Monnerat, Severine; Cestari, Igor; Kalidas, Savitha; Li, Qiong; Regmi, Sandesh; Nguyen, Suong; Brown, Rob; Anupama, Atashi; Parsons, Marilyn; Phillips, Meg; Stuart, Ken*

179 Computational analyses of alternative trans-splicing in Trypanosoma brucei

Wang, Zefeng; *Tsai, Yi-Hsuan*

180 Development and characterization of inhibitors against essential trypanosome hexokinases

Harris, Michael T.; *Golden, Jennifer; Aubé, Jeffrey; Schroeder, Chad; Joice, April; Maselli, Andrew; Morris, James*

181 Optimizing RNAi activity for study of the Leishmania flagellum

Lye, Lon-Fye; *Fowlkes, Tiffanie; Brettmann, Erin; Owens, Katherine; Clipperton, Elizabeth; Marcus, Joseph; Beverley, Stephen M*

182 Does Hemoglobin-Induced Peroxidation of Trypanosome Lytic Factor-1 Lead to Trypanosome Lytic Factor-2 Biogenesis?

Styer, Amy L.; *Hajduk, Stephen*

183 Repurposing human PDE4 inhibitors as a starting point for trypanosomal drug discovery

Marine Biological Laboratory; *Gustafson, Alden; Ochiana, Stefan; Amata, Emanule; Woodring, Jennifer; Wang, Cuihua; Pollastri, Michael; Campbell, Robert*

184 Comparative Genomics of mRNA metabolism pathways in Eukaryotes

Bannerman-Chukualim, Bridget; *Carrington, Mark*

185 New Tools for Studying Post-Transcriptional Regulation in Kinetoplastid Parasites

Yates, Phillip A.; *Soysa, Radika; Carter, Nicola*

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04/24/2013

187 Mucin-Like Associated Surface Protein: Potential Vaccine Candidate against Chagas Disease
Serna, Carylinda

188 Structure-function analysis of the novel chromosomal passenger complex in Trypanosoma brucei
***Li, Ziyin**; Yu, Zhonglian; Liu, Yi; Wang, Tao*

189 The mitochondrial calcium uniporter of Trypanosoma brucei is essential for growth and infectivity
***Huang, Guozhong**; Vercesi, Anibal; Docampo, Roberto*

190 A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in *Leishmania donovani*.
***Martin, Jessica L.**; Yates, Phillip; Cassera, Maria Belen; Boitz, Jan; Fulwiler, Audrey; Ullman, Buddy; Carter, Nicola*

191 Metabolic Reprogramming During Purine Stress in the Protozoan Pathogen Leishmania donovani
***Carter, Nicola**; Martin, Jessica; Yates, Phil; Soysa, Radika; Myler, Peter; Ramasamy, Gowthaman; Yang, Feng; Burnum-Johnson, Kristen; Petyuk, Vladislav; Camp, David; Smith, Richard; Wilmarth, Phillip; David, Larry*

192 BioID for trypanosomes - novel bilobe components identified by proximity to TbMORN1
***Morriswood, Brooke**; Havlicek, Katharina; Demmel, Lars; Yavuz, Sevil; Sealey-Cardona, Marco; Vidilaseris, Keni; Kostan, Julius; Djinovic-Carugo, Kristina; Roux, Kyle; Warren, Graham*

193 Trypanosoma brucei RAP1 plays an important role in suppressing subtelomeric VSG associated gene conversion
***Nanavaty, Vishal P.**; Li, Bibo*

194 Independent signalling mechanisms act to regulate life-cycle differentiation in Trypanosoma brucei
***Szoor, Balazs**; Ruberto, Irene; Dyer, Naomi; Acosta Serrano, Alvaro; Matthews, Keith R*

195 The essential Leishmania major MAP kinase LmaMPK4 is involved in pH sensing and differentiation of metacyclic promastigotes
***Dacher, Mariko**; Morales, Miguel A.; Pescher, Pascale; Leclercq, Olivier; Beverley, Stephen M.; Späth, Gerald F.*

196 RET1-DSS1 complex is required for gRNA maturation
***Suematsu, Takuma**; Aphasizheva, Inna; Huang, Lan; Aphasizhev, Ruslan*

197 MITOCHONDRIAL CHAPERONE AND KDNA
***Tyc, Jiri**; Skalicky, Tomas; Basu, Somsuvro; Lukes, Julius*

198 Detecting and defining subdomains at the surface of African trypanosomes by proteomics
***Gadelha, Catarina**; Zhang, Wenzhu; Chait, Brian T.; Field, Mark C.*

199 Rab11 Regulates Glycosylphosphatidylinositol (GPI)-anchor Protein Trafficking to the Plasma Membrane by an Unconventional Pathway Involving the Contractile Vacuole of Trypanosoma cruzi
***Niyogi, Sayantanee**; Mucci, Juan; Campetella, Oscar; Docampo, Roberto*

200 Simultaneous transcriptome profiling of Trypanosoma cruzi parasites and human host cells
***Li, Yuan**; Caradonna, Kacey; Choi, Jungmin; Padmanabhan, Prasad; Temanni, Mohamed; Corrada Bravo, Hector; El-Sayed, Najib; Burleigh, Barbara*

201 On Being the right size -Trypanosoma brucei Sec16 and ER exit sites
***Sealey, Marco**; Warren, Graham*

202 Inhibiting the essential FoF1-ATPase activity in the infectious form of Trypanosoma brucei
***Panicucci, Brian P.**; Walker, John; Zikova, Alena*

203 The cooperative roles of PHO80-like cyclins in regulating the G1/S transition and posterior cytoskeletal morphogenesis in Trypanosoma brucei
***Liu, Yi**; Li, Ziyin*

204 Distinct PPR proteins are responsible for coupling of mRNA editing, polyadenylation and translation in mitochondria of trypanosomes
***Afasizheva, Inna**; Maslov, Dmitri; Huang, Lan; Afasizhev, Ruslan*

205 High-throughput screening of a kinase-targeted library at the GlaxoSmithKline OpenLab: Identification and characterization of thousands of anti-trypanosomal hit compounds and opportunities for "open-source" lead optimization.
***Pollastri, Michael P.**; Diaz, Rosario; Luengo-Arratta, Sandra; Seixas, João D.; Berlanga, Manuela; Colmenarejo, Gonzalo; Fiandor, Jose Maria; Gonzalez, Silvia; Manzano, Pilar; Martin, Jose Julio; Navarro, Miguel*

206 Immunolocalization of the virulence factor NTPDase-1 in Trypanossoma cruzi
***Oliveira, Cláudia**; Mariotini-Moura, Christiane; Castro, Felipe; Trindade, Mellina; Bastos, Matheus; Baqui, Munira; Almeida, Márcia; Fietto, Juliana*

207 Insights into expression of GPEET procyclin during differentiation from early to late procyclic form T. brucei
***Knüsel, Sebastian**; Roditi, Isabel*

208 The bi-lobe associated TbLRRP1 is a RanGTPase activating protein
***Brasseur, Anais**; Bayat, Shima; Zhou, Qing; He, Cynthia, Y*

209 p24 transmembrane proteins regulate early secretory trafficking in African trypanosomes
***Kruzel, Emilia K.**; Lowe, Tiffany; Bangs, James*

210 RNA Polymerase II Transcription of the Serum Resistance Associated Protein gene containing Expression Site in Trypanosoma brucei rhodesiense
***Kieft, Rudo**; Chandler, Chelsey; Murtha, Jacqueline Ann; Hajduk, Stephen L.*

211 Action of Trypanosome Lytic Factor Against Leishmania major
***Nelson, Maria**; Samanovic-Golden, Marie; Raper, Jayne*

212 Transforming a Parasite into an Antigen Display Platform
***Pinger, Jason**; Stavropoulos, Pete; Papavasiliou, F. Nina*

213 Identification and functional analysis of a GPR89 homologue that promotes the development of stumpy forms in Trypanosoma brucei
***Rojas, Federico**; Milne, Rachel; Thompson, Joanne; Matthews, Keith R*

214 DNA double strand break repair in Trypanosoma brucei: reaction kinetics and factors
***Prorocic, Marko M.**; McCulloch, Richard; Trenaman, Anna*

215 Mechanism of flagellum construction and maintenance
***Santi-Rocca, Julien**; Blisnick, Thierry; Bastin, Philippe*

216 Investigating the role of the *Leishmania (L.) major* HASPs and SHERP genes during metacyclogenesis in the sand fly vectors *Phlebotomus papatasi* and *P. duboscqi*
***Doehl, Johannes**; Sádlová, Jovana; Volf, Petr; Smith, Deborah F.*

217 Homologue of a human mitochondrial deadenylase is a cytosolic ribonuclease in T. brucei
***Zimmer, Sara L.**; Sakyiama, Joseph; Read, Laurie*

218 TbISWI and NLP are part of a novel ISWI complex in T. brucei
***Witmer, Kathrin**; Narayanan, Mani Shankar; Stanne, Tara; Ling, Alexandra; Wiesler, Simone; Bauer, Rebekka; Kushwaha, Manish; Rudenko, Gloria*

219 Trypanosoma brucei Polo-like kinase: function and regulation
***Thomas, Elizabeth**; May, Sophie; Hammarton, Tansy*

220 Identification and Comparison of polymorphisms in the Trypanosoma cruzi Complement Regulatory Protein (Tc-CRP) encoding gene from different parasite strains
***Meira, Wendell SF**; Paiva, Priscila; Marques, Tatiane; Lages-Silva, Eliane; Ramirez, Luis; Norris, Karen*

221 Investigating T. brucei oligosaccharyltransferase STT3A and STT3B sequon specificity in vivo.

***Jinnelov, Anders**; Ferguson, Michael*

222 An endossomal eIF2alpha kinase is activated by phosphorylation during reservosomes depletion in Trypanosoma cruzi
***Augusto, Leonardo S.**; Moretti, Nilmar S.; Schenkman, Sergio*

223 TbTim62: A novel component of the mitochondrial inner membrane protein translocase in Trypanosoma brucei.
SINGHA, UJJAL K.

224 Structure and Function of Trypanosoma cruzi Flagellar Calcium Binding Protein
***Olson, Cheryl L.**; Maric, Danijela; Asfaw, Sofya; Buchanan, Kathryn; Engman, David*

225 Evaluation of safety and immugenicity of live attenuated Leishmania donovani p27 and Centrin gene deleted parasites in hamster model using intradermal route of immunization
***Araujo Fiuza, Jacqueline**; Dey, Ranadhir; Gannavaram, Sreenivas; Davenport, Dwann; Duncan, Robert; Nakhasi, Hira*

226 In vitro screening of compounds identifies RNA editing inhibitors
***Mehta, Vaibhav N.**; Moshiri, Houtan; Salavati, Reza*

227 Computational recognition of cis-regulatory elements in trypanosomatids
***Gazestani, Vahid**; Salavati, Reza*

228 Open chromatin of the VSG active Expression Site is maintained independently of transcription in Trypanosoma brucei
***Aresta Branco, Francisco**; Pimentel, Mafalda; M. Figueiredo, Luísa*

229 TriTrypDB: the functional genomics resource for kinetoplastids
***Harb, Omar S.**; Brunk, Brian; Hertz-Fowler, Christiane; Silva, Fatima; on behalf of the Kinetoplastid Database Consortium, -*

230 The role of POLIC arginine methylation in Trypanosoma brucei kDNA replication.
***Miller, Jonathan C.**; Concepcion-Acevedo, Jeniffer; Dodard, Garvin; Read, Laurie; Klingbeil, Michele*

231 In silico Targeting of Trypanosomal RNA Editing Ligase 1 for Drug Discovery
***Allum, Fiona**; Mehta, Vaibhav; Sulea, Traian; Salavati, Reza*

232 Insertion of the Leishmania donovani peroxin-5 into glycosomal membranes.
***Davidsen, Amanda E.**; Jardim, Armando*

233 Descriptive and comparative analysis of the global transcriptome response of Trypanosoma cruzi to different medium conditions

***Probst, Christian M.**; Leprovst, Felipe; Preti, Henrique; Krieger, Marco Aurelio*

234 TrypanoGEN: an integrated approach to the identification of genetic determinants of susceptibility to trypanosomiasis
***Hertz-Fowler, Christiane**; MacLeod, Annette; The TrypanoGEN, Network*

235 A proteomics analysis of the trypanosome transition zone
***Dean, Samuel**; Gull, Keith*

236 The role of an HMG box-containing proteins, TbKAP4, in Trypanosoma brucei kDNA
wang, jianyang

237 Characterization of a novel Leishmania aminopeptidase implicated in parasite virulence
***Norris, Brianna**; Vanderkolk, Kaitlin; Vacchina, Paola; Joyce, Michelle; Morales, Miguel*

238 Control of Protein Expression in Trypanosoma brucei
***Kelner, Anna**; Cowling, Victoria; Ferguson, Michael*

239 The roles of 3'-exoribonucleases and the exosome in trypanosome mRNA degradation
***Fadda, Abeer**; Färber, Valentin; Droll, Dorothea; Clayton, Christine*

Talk Session I: Cell Biology I
(Chair) Kent Hill

04/21/2013

1A Genome wide dissection of the quorum sensing signaling pathway in Trypanosoma brucei

M. Mony, Binny (Centre for Immunity, Infection and Evolution, University of Edinburgh, UK.)*; MacGregor, Paula (Centre for Immunity, Infection and Evolution, University of Edinburgh, UK.); Cowton, Andrew (Centre for Immunity, Infection and Evolution, University of Edinburgh, UK.); Ivens, Alasdair (Centre for Immunity, Infection and Evolution, University of Edinburgh, UK.); Horn, David (London School of Hygiene and Tropical Medicine, UK.); R. Matthews, Keith (Centre for Immunity, Infection and Evolution, University of Edinburgh, UK.)*

Trypanosoma brucei, the protozoan flagellate that is responsible for African sleeping sickness in humans and Nagana in cattle, exhibits pleomorphism in the mammalian blood. There exist two major forms- the proliferative slender form and the growth arrested, transmissible, stumpy form. The slender to stumpy transition is density dependent and is crucial for the life cycle of the parasite, ensuring both control of infection, chronicity and transmission. The differentiation is triggered by a proposed ‘stumpy induction factor’ (SIF), although its identity and intracellular signaling pathway is uncharacterized. Laboratory adapted (monomorphic) strains cannot respond to SIF, but can undergo an incomplete differentiation to stumpy-like forms after exposure to cell permeable hydrolysable cAMP and AMP analogues. We sought to explore this differentiation process by adopting a forward genetic approach using a genome wide RNAi library screen in monomorphs. In separate screens, monomorphic parasites were exposed to cell permeable cAMP or AMP analogues to select cells that remained proliferative and so were unresponsive to these signals. This reproducibly identified a cohort of genes implicated in all steps of the signaling pathway, from cAMP metabolism, through signal transducers (kinases, phosphatases) to gene expression regulators (RNA binding proteins). The identified genes were validated through independent transfections in monomorphs and have been validated in pleomorphic cells for their role in SIF-induced stumpy formation. This approach has provided a comprehensive dissection of the stumpy induction pathway in T. brucei and presents scope for identifying the intersection between cAMP/AMP signaling and SIF induced differentiation in Trypanosomes.

1B An RNAi library screen identifies proteins that sensitize African trypanosomes to elevated cAMP, suggesting a novel signaling pathway

Bachmaier, Sabine (University of Munich (LMU), Biocenter, Section Genetics, Martinsried, Germany)*; Gould, Matthew K. (Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK); Ali, Juma A. M. (Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK); Munday, Jane (Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK); Horn, David (Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK); de Koning, Harry P. (Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK); Boshart, Michael (University of Munich (LMU), Biocenter, Section Genetics, Martinsried, Germany)*

The signaling molecule cAMP plays crucial regulatory roles in almost all eukaryotic cells. In T. brucei, the genetic or pharmacological manipulation of the intracellular cAMP concentration results in severe cytokinesis phenotypes with subsequent cell death (Salmon et al., 2012; de Koning et al., 2012). Consequently, the cAMP-specific phosphodiesterases have been validated as excellent drug targets. However, the major downstream target of cAMP, the cAMP-dependent protein kinase (PKA), is not activated by cAMP, nor have homologs of other known mammalian cAMP effectors been identified. We thus used genome-wide RNAi library screening (Alsford et al., 2011) to select cells resistant to CpdA, a novel and highly specific PDE inhibitor (de Koning et al, 2012), which kills bloodstream trypanosomes via elevated intracellular cAMP. Four candidate genes (CARP1-4: cyclic AMP response proteins) were identified, whose depletion confers different degrees of resistance to CpdA. CARP1, a protein unique to kinetoplastid parasites has two predicted cNMP binding domains, and its depletion resulted in up to 200-fold CpdA resistance. We suggest that this protein is a primary cAMP sensor and CARP2-4 proteins may be components of a novel cAMP signaling pathway. Binding of cAMP to CARP1, physical and genetic interactions among the CARP proteins, and their subcellular localisation are under investigation. The novel kinetoplast-specific cAMP signalling cascade might qualify as a promising new drug target for Human African Trypanosomiasis. Alsford et al (2011) Genome Res. 21:915-924 De Koning et al (2012) J. Infect. Dis. 206:229-237 Salmon et al (2012) Mol. Microbiol. 84:225-242

1C Role of the Trypanosoma brucei BBSome in host-pathogen interaction

***Langousis, Gerasimos (UCLA, Dept of MIMG)**; Hill, Kent L. (UCLA); Shimogawa, Michelle (UCLA, Dept of MIMG); Edwin, Saada (UCLA, Dept of MIMG); Ng, Eva (UCLA, Dept of MIMG)*

Trypanosoma brucei are noxious parasites afflicting mammals in sub-Sahara Africa. The human disease, sleeping sickness, is fatal if untreated. A central feature of trypanosome biology is the parasite's single flagellum, an essential organelle that ensures cell propulsion, morphogenesis and cytokinesis. Moreover, the flagellar membrane is a critical host-pathogen interface. Flagellum-mediated host-pathogen interactions are postulated to rely on membrane proteins that dynamically localize at the flagellum and modulate infection and virulence. We sought to test this hypothesis by studying the BBSome, a multimeric complex of BBS (Bardet Biedl Syndrome) proteins that mediates protein trafficking to/from the flagellar membrane. In vertebrates, nematodes and green algae, disruption of the BBSome perturbs flagellum protein homeostasis and causes defects in flagellum-dependent signaling without generally disrupting flagellum assembly. By employing glycerol gradient centrifugation and blue native gel electrophoresis, we discovered the existence of a BBSome-like protein complex in the mammalian-infectious form of T.brucei. We show that this complex localizes at the base of the flagellum, consistent with a role in controlling flagellar homeostasis and signaling. In order to test the role of the BBSome in T.brucei infectivity we produced genetic knockouts of select BBS genes. These null mutants are viable in culture without any gross alteration in cell morphology or growth rate. BBS null mutants show attenuated infectivity and virulence in a mouse model of infection, indicating a crucial role of flagellum signaling in host-pathogen interactions. Determining the composition of the T. brucei BBSome and disclosing the BBSome-modulated pathways are underway. We expect our studies will advance efforts to exploit the trypanosome flagellum as a drug target and provide insight into fundamental aspects of eukaryotic biology.

1D The global view: Characterisation of organelle segregation and distribution throughout the T. brucei bloodstream form cell cycle.

Vaughan, Sue (Oxford Brookes University)*; Hughes, Louise (Oxford Brookes University); Towers, Katie (Oxford Brookes University); Barry, Samantha (Oxford Brookes University); Starborg, Tobias (University of Manchester); Gull, Keith (University of Oxford)*

Serial block face scanning electron microscopy (SBFSEM) is a new technique that enables visualization of large volumes containing numerous cells at different cell cycle stages. This technique provides a whole cell view whilst at the same time revealing internal ultrastructural detail of organelle morphology and distribution. The T. brucei cell has a long tubular shape with a single flagellum. Some organelles, present as single copy or in low copy number are asymmetrically localised to certain regions of the cell such as the flagellum, flagellar pocket, basal body, kinetoplast, nucleus and Golgi. The mitochondrion and endoplasmic reticulum are distributed throughout the cell body, as are multi-copy organelles such as glycosomes and acidocalciosomes. Whole cell volumes at different stages of the cell division cycle were extracted and all major organelles and cellular structures were modeled and quantified. This allowed a complete view of the temporal order and spatial positioning in which each organelle duplicated, segregated or assembled. New flagellum length, kinetoplast and nuclear division, acknowledged cell cycle stage markers, could now be placed in context with other cell organelles. Our data included the discovery of novel architecture at the tip of the growing new flagellum, further extension of our understanding of mitochondrial division and new features of nuclear division. Our 3D global modeling provides cartographic snapshots combining spatial information with quantitative volumetric analyses of all major organelles across the cell division cycle.

1E Identification of kinetochore proteins in Trypanosoma brucei

Akiyoshi, Bungo (University of Oxford)*; Gull, Keith (University of Oxford)*

Faithful transmission of genetic material is essential for the survival of all organisms. Eukaryotic chromosome segregation is driven by the kinetochore, the macromolecular protein complex that assembles on centromeric DNA to capture spindle microtubules and move chromosomes. To date, kinetochores have only been studied in popular eukaryotes (e.g. yeasts, worms, flies, and human) that are closely related in an evolutionary timescale, and it is therefore not known whether all eukaryotes use the same segregation mechanisms. The evolutionary origin(s) of kinetochores also remains unknown. To gain insights into these questions, we work on Trypanosoma brucei, an experimentally-tractable kinetoplastid parasite that branched early in eukaryotic history. T. brucei has been shown to segregate a series of megabase, intermediate and minichromosomes. Whilst an intranuclear spindle is formed and kinetochore-like structures are seen via electron microscopy, no canonical kinetochore protein has been identified in kinetoplastids and the segregation mechanism remains unclear. To reveal the mechanism of chromosome segregation in T. brucei, we performed a localization-based screening and proteomics approach, leading to the identification of 19 proteins that show dynamic localization patterns expected for kinetochore proteins. Consistent with this, RNAi-mediated knockdown results in severe chromosome mis-segregation. These proteins are well conserved among kinetoplastids (e.g. T. cruzi, Leishmania, and Bodo saltans). However, they bear no detectable homology to kinetochore proteins in other eukaryotes, raising a possibility that kinetoplastids use a chromosomal segregation mechanism involving novel components.

1F Kharon1 mediates flagellar targeting of a glucose transporter in Leishmania mexicana

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Flagellar membrane proteins play important roles in the biology of Kinetoplastid parasites such as Leishmania and Trypanosoma species. Analysis of individual membrane proteins and of the trypanosome flagellar membrane proteome have identified flagellar membrane proteins likely to be involved in signal transduction, including potential protein kinases, adenylate cyclases, and Ca2+ channels. Because the flagellum can serve as a platform for sensing and signal transduction, there is great interest in elucidating the mechanisms that target integral membrane proteins to this organelle. The glucose transporter LmxGT1 is selectively targeted to the flagellar membrane in Leishmania mexicana parasites. Previous analysis of LmxGT1 revealed a small flagellar targeting domain sufficient to target other integral membrane proteins to the flagellar compartment. Here we report the identification of a novel protein, unique to Kinetoplastida and designated Kharon1, which interacts with the previously identified flagellar targeting domain of LmxGT1 and is required for targeting this permease to the flagellar membrane. Kharon1 null mutants are strongly impaired in the flagellar targeting of LmxGT1, and trafficking of the permease was arrested in the flagellar pocket. Interestingly, flagella biogenesis appears unaffected in Kharon1 null mutants. Immuno-localization of tagged Kharon1 revealed that it is located in the flagellar pocket where membrane proteins are probably sorted. Kharon1 is the first protein identified in Kinetoplastid protozoa that selectively targets a polytopic membrane protein to the flagellar compartment. Current work aims to identify other components of the Kharon1 machinery as well as other membrane proteins that traffic to the flagellar compartment in a Kharon1 dependent manner.

1G A specific life-cycle stage of Trypanosoma brucei exhibits social motility

Imhof, Simon*; Roditi, Isabel*

When Trypanosoma brucei procyclic forms are pipetted onto the surface of an agarose plate, they exhibit social motility (SoMo) in a manner reminiscent of bacteria such as Myxococcus xanthus. After a few days incubation, parasite communities form “fingers” that radiate out from the site of inoculation on the plate and are capable of sensing and avoiding each other. We hypothesized that the parasites’ behaviour on plates might correspond to an event in their life cycle. At least two migrations occur in the tsetse fly: first, the movement by procyclic forms from the midgut lumen into the ectoperitrophic space, and second, the migration from the ectoperitrophic space across the cardia to the salivary glands. The trypanosomes found in the midguts of freshly infected flies (early procyclic forms) express two major proteins on their surface, the GPI-anchored procyclins EP and GPEET. When the parasite reaches the ectoperitrophic space, GPEET is down-regulated and the cells express only EP (late procyclic forms). At present GPEET is the only marker to distinguish between early and late procyclic forms. We were able to show that SoMo is a property of early procyclic forms and that trypanosomes become SoMo-negative when they differentiate into late procyclic forms. GPEET is not essential for SoMo, however, because GPEET null mutants are still SoMo-positive. This indicates that other differentially regulated proteins are likely to be responsible for the SoMo phenotype. Several mutants that show defects in establishing salivary gland infections exhibit normal SoMo. These results suggest that SoMo corresponds to an early event in midgut infection, most probably the migration from the lumen to the ectoperitrophic space.

1H Paratrypanosoma – the earliest branch of trypanosomatids and the changing biodiversity landscape of the monoxenous parasites

Lukes, Julius (Biology Center)*; Votypka, Jan (Charles University); Flegontov, Pavel (Biology Center); Skalicky, Tomas (Biology Center); Tyc, Jiri (Biology Center); Jirku, Milan (Biology Center); Yurchenko, Vyacheslav (University of Ostrava); Archibald, John M. (Dalhousie University); Maslov, Dmitri A. (University of California)*

Monoxenous trypanosomatids are frequent parasites of insects worldwide. As compared to their dixenous relatives, much less is known about their host specificity, diversity and phylogeny, with the genus level taxonomy remaining artificial and species criteria elusive. Yet the dixenous Leishmania and Phytomonas clearly emerged from within the monoxenous trypanosomatids of dipteran and hemipteran hosts testifying to the importance of this group for understanding the origin and evolution of parasitism in the entire family. In frame of our studies of insect trypanosomatids, we have isolated a flagellate, named here Paratrypanosoma confusa, which constitutes a distinct branch between free-living Bodo saltans and obligatory parasitic genus Trypanosoma indicating that even this evolutionarily successful and widespread genus evolved from an insect-dwelling ancestor. The branching of Paratrypanosoma is strongly supported by rRNA gene and >100 individual protein gene phylogenies together with analyses of concatenated datasets. We and others have initiated the exploration of insect trypanosomatid biodiversity by defining typing units (molecular species), of which near 130 has been discovered recently in hemipteran and dipteran hosts from all continents except Antarctica. We have also defined natural taxonomic subdivisions within this species-rich family. The phylogenomics studies of these major groups will have a strong impact on our understanding of the emergence and success of dixenous parasitism in kinetoplastids.

Talk Session II: Nucleus and Kinetoplast

(Chair) Sergio Schenkman

04/22/2013

2A Genome-wide organisation of DNA replication initiation in kinetoplastid parasites

McCulloch, Richard (University of Glasgow)*; Dickens, Nicholas (University of Glasgow); Marques, Catarina (University of Glasgow); Marcello, Lucio (University of Glasgow)*

DNA replication is central to the propagation of life and initiates by the designation of genome sequences as origins; sites where DNA synthesis begins. Eukaryotic linear chromosomes are replicated from potentially thousands of origins, which are designated by binding of the Origin Recognition Complex (ORC) to genome sites that rarely display sequence conservation. In Trypanosoma brucei we have mapped replication origins in the nuclear genome through next generation sequencing, comparing read depth in DNA from S and G2 phase cells. This revealed ~40 origins, a remarkably small number for a eukaryote, all of which flank the multigene transcription units in the core of this genome. Moreover, we find that the T. brucei origins represent only a fraction of the mapped binding sites for one ORC factor (ORC1/CDC6), and that some origins initiate replication more efficiently than others. These data suggest that origin redundancy and a temporal order of firing are found in T. brucei , phenomena in common with replication initiation in other eukaryotes but poorly understood. To examine this, we have mapped origins in the genome of Leishmania major , which displays considerable gene syntenty with T. brucei, but also notable structural differences: chromosome numbers and size are different between the parasites, as is ploidy stability. We find a very similar number of total origins in L. major compared with T. brucei , and many localise to syntenic regions, suggesting conservation of origin features. However, origin organisation appears strikingly different: we find only a single origin in each of the 36 L. major chromosomes, and each origin appears to fire with equal efficiency, suggesting replication adaptations that reflect the different genome architectures.

2B An in vitro nucleosome reconstitution system to study chromatin modification by trypanosome DOT1 methyltransferases

***Dindar, Guelcin**; Mehlhorn, Christine; Schneider, Ludmila; Anger, Andreas; Hake, Sandra; Janzen, Christian*

Reorganization of chromatin structure regulates many basic biological processes like DNA repair, transcription regulation and cell differentiation. In recent years, many post-translational histone modifications (PTMs) have been identified that influence chromatin structure and nuclear architecture. First direct evidence that histone PTMs are involved in the differentiation process in T. brucei was obtained when we characterized two histone methyltransferases of the DOT1 family. DOT1 proteins are evolutionarily conserved histone H3 lysine 79 (H3K79) methyltransferases that are involved in heterochromatin formation and cell development. T. brucei has two DOT1 homologues, DOT1A and DOT1B, which are responsible for di- and tri-methylation of H3K76, respectively (H3K76 in T. brucei is homologous to H3K79 in other organisms). Surprisingly, we found that methylation of H3K76 by DOT1B is essential for developmental differentiation of bloodstream forms to procyclic forms. To analyze how DOT1B influences changes in chromatin structure during differentiation, we established a nucleosome reconstitution system with individually purified components. The enzymatic activities of the different DOT1 enzymes in this system reflect what we observed in vivo, which allows us to elucidate the molecular basis for specificity and activity of DOT1 histone methyltransferases under defined in vitro conditions. Based on structural homology modeling, we identified amino acids within the proposed lysine binding pocket of the enzyme that appear to be responsible for the tri-methylation activity of DOT1B. We now plan to use this system to identify proteins that bind to methylated H3K76 to unravel the mechanisms behind changes of chromatin structure during developmental differentiation of African trypanosomes.

2C Uncovering evolution in 3D: Architecture of the trypanosome nuclear pore complex reveals conserved and divergent features across a billion years

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The nuclear pore complex (NPC) is the ubiquitous assembly that mediates transport between the eukaryotic nucleus and cytoplasm. Both the NPC and nuclear envelope (NE) serve as structural and organizational platforms from which intranuclear compartments and the nuclear lamina are built. These structures provide key regulatory functions for gene expression via influencing nuclear architecture, acting as control points for various nuclear functions. The NPC is a large (50-100 MDa), cylindrical octagonally symmetric complex comprising ~500 polypeptides (nucleoporins) from ~30 different proteins. Detailed NPC/NE composition and structural information is restricted to the closely related opisthokonts, the animals and fungi; therefore we have examined the NPC and NE in a highly divergent eukaryote, the trypanosome Trypanosoma brucei. Understanding trypanosome NPC/NE functions requires a detailed description of both the NPC and NE protein interactomes. Using GFP as an affinity handle and a novel high-pressure cryomilling protocol we affinity isolated sub-complexes of nucleoporins as well several putative NE proteins, identifying interactions between a large cohort of nucleoporins to build a structural map of the NPC and its neighbors in the NE. We report a complex picture of remarkably conserved core scaffold NPC subcomplexes, which likely form a stable structural anchor. However, key peripheral structural modules present in opisthokonts seem to be absent, most prominently the trans-membrane anchors. Finally we provide robust evidence linking the NPC to the recently described nuclear lamina and additional NE components. We present evidence that elaborate core structures of the NPC and even NE may have been established prior to the radiation of the eukarya from a common ancestor.

2D TbNMD3 regulates mRNA via an Xpo1-linked nuclear export mechanism.

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In Trypanosoma brucei TbNMD3, a homologue of eukaryotic NMD3, has been implicated in rRNA processing and export, consistent with the function of this molecule in other organisms. Interestingly, we have also discovered that TbNMD3 RNAi generates a strong upregulation of PAG transcripts, genes co-transcribed within the procyclin gene locus. RNAseq transcriptome analysis of TbNMD3-depleted cells show that this effect is highly specific whereas reporter assays demonstrate that this effect is dependent upon the PAG gene 5'UTR and restricted to the mRNA level (no protein elevation occurs). ChIP analysis confirmed the PAG mRNA up-regulation is not mediated by enhanced locus-specific transcription, nor is it a consequence of translational inhibition resulting from the effects of TbNMD3 depletion on rRNA maturation. Importantly, depletion of the 7SL RNA nuclear export factor, XPO1, recapitulates the effects of TbNMD3 depletion on PAG mRNAs. Moreover, in situ hybridisation analyses demonstrate a bulk nuclear accumulation of all mRNAs in response to TbNMD3 depletion. This invokes a novel mRNA regulatory mechanism involving the NMD3- and XPO1-dependent nuclear export of mRNA cargos, with PAG mRNA accumulation being caused by its nuclear sequestration and hence protection from rapid cytoplasmic turnover. This is the first nuclear export pathway for mRNA identified in kinetoplastid parasites and this novel function for NMD3 may be conserved in other systems.

2E The telomere DNA binding activity of Trypanosoma brucei TRF is important for suppression of sub-telomeric VSG switching

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Trypanosoma brucei, the causative agent of human African trypanosomiasis (HAT), effectively escapes the host immune response by regulating the expression of its surface antigen, Variant Surface Glycoprotein (VSG), via monoallelic expression and by regularly switching its expressed VSG via VSG switching. VSGs are expressed exclusively from VSG expression sites located adjacent to telomeres, which are nucleoprotein complexes at the natural chromosome ends. We have identified TbTRF as a telomere DNA binding factor, which is essential for maintaining the telomere end structure. In this study we report that transient depletion of TbTRF leads to a significant increase in the VSG switching frequency, and many switchers arose through the in-situ switching pathway that is seldom used in WT cells. To investigate the underlining molecular mechanism, we focused on the telomere DNA binding activity of TbTRF and solved the NMR structure of its myb domain, which harbors a helix-turn-helix motif conserved with the hTRF1 myb domain. The association of TbTRF with the telomere in T. brucei cells was not affected by the heavily modified J residue (b-D-glucosyl-hydroxymethyluraci) usually found in T. brucei telomeres. However, several point mutations in the TbTRF myb domain were identified to exhibit weakened DNA binding activity both in vitro and in vivo. The same mutants also led to an elevated VSG switching frequency, although gene conversion is still the predominant switching mechanism in these mutants as in WT cells. Our data indicate that the DNA binding activity of TbTRF is critical for VSG switching regulation.

2F Antigenic variation in T. brucei: evidence for VSG allelic exclusion through sequence-specific genetic interference

Hutchinson, Sebastian (LSHTM)*; Alsford, Sam (LSHTM); Horn, David (LSHTM)*

Antigenic variation in African trypanosomes depends upon allelic exclusion of Variant Surface Glycoprotein (VSG) expression sites (ESs) at telomeres and is essential for persistence in the mammalian host. The mechanisms controlling this VSG exclusion remain poorly understood. We are investigating a role for VSG expression-site derived transcripts in maintaining monoallelic VSG expression. Initially, we found that blocking VSG-ES transcription (using a tetracycline repressor) induced high frequency antigenic variation, indicating repression of silent VSGs by the active VSG. Next, using telomere-mediated fragmentation to engineer reporter cassettes adjacent to de novo telomeres, we observed genetic interference among homologous sequences; this interference can repress VSG, GFP or selectable marker gene expression. Specifically, a recombinant VSG can repress the native VSG, and a GFP gene can repress an NPT gene with a common untranslated region. Using RNA interference (RNAi) to manipulate transcript levels, we demonstrate that knockdown of telomeric transcripts triggers derepression of other telomeric transcripts. We propose a model whereby sequence-specific genetic interference controls VSG allelic exclusion.

2G Base J Insertion and Function in Leishmania

***Baugh, Loren**; Borst, Piet; Genest, Paul-Andre; Myler, Peter J.; van Luenen, Henri*

β-glucosylhydroxymethyluracil (base J) is a modified DNA base thus far found only in Euglenozoa. Base J synthesis requires two enzymes (JBP1 and JBP2) that catalyze hydroxylation of thymine, and the resultant HOMeU is modified by a putative glucosyltransferase. We have previously shown that knock-out of JBP2 in Leishmania caused gradual loss of J, transcriptional read-through at convergent strand-switch regions (cSSRs) that contain internal J (iJ), as well as false-starts at transcription initiation sites. Bromodeoxyuridine (BrdU) treatment of JBP2KO parasites caused further reduction in iJ levels, and eventual death of the cells. We have now used RNA-seq and Nanostring technology to quantify mRNA levels in BrdU-treated WT L.□tarentolae and a new JPB2KO line, revealing consistent and substantial changes in expression of several genes near iJ sites. These results suggest that loss of iJ induces de-repression of genes deleterious to cell growth, and/or down-regulation of essential genes (due to accumulation of antisense RNA at cSSRs). To investigate the signals responsible for J insertion, we cloned several J-containing sequences into plasmids and grew them episomally in L.□tarentolae. Plasmids containing cSSR-25.2 sequence or the telomeric hexamer sequence ([GGGTTA]10) accumulated J when grown in WT but not JBP2KO cells, while those containing the atypical cSSR-28.2 (which lacks iJ in WT cells) accumulated no detectable J. SMRT sequencing of the J-containing plasmids revealed that most J sites occurred in pairs at 13-nt intervals on opposite DNA strands. Thus, we hypothesize that JBP2 recognizes the signal for de novo J insertion, while JBP1 is responsible for J maintenance (following DNA replication) by recognizing this J and inserting a new J downstream on the opposite strand.

2H A trans-spliced telomerase RNA dictates telomere synthesis in Trypanosoma brucei

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Telomerase is a ribonucleoprotein enzyme typically required for sustained cell proliferation. Although both telomerase activity and the telomerase catalytic protein component, TbTERT, have been identified in the eukaryotic pathogen Trypanosoma brucei, the RNA molecule that dictates telomere synthesis has remained unknown. Here we identify the RNA component of T. brucei telomerase, TbTR, and provide phylogenetic and in vivo evidences for TbTR's native folding and activity. We show that TbTR is processed through trans-splicing, is a capped transcript that interacts and co-purifies with TbTERT in vivo. Deletion of TbTR caused progressive shortening of telomeres at a rate of 3–5 bp/PD, which can be rescued by ectopic expression of a wild type allele of TbTR in an apparent dose-dependent manner. Remarkably, introduction of mutations in the TbTR template domain resulted in corresponding mutant telomere sequences, demonstrating that telomere synthesis in T. brucei is dependent on TbTR. We also propose a secondary structure model for TbTR based on phylogenetic analysis and chemical probing experiments, thus defining TbTR domains that may have important functional implications in telomere synthesis. Identification and characterization of TbTR not only provide important insights for investigation of T. brucei telomere functions, which have been shown to play important roles in T. brucei pathogenesis, but also offer T. brucei as an attractive model system for studying telomerase biology in pathogenic protozoa and for comparative analysis of telomerase function with higher eukaryotes.

2I Control of Trypanosoma brucei kDNA replication by the TbHSLVU Protease

Tiengwe, Calvin (The Johns Hopkins University School of Medicine)*; Englund, Paul T (The Johns Hopkins University School of Medicine); Jensen, Robert E (The Johns Hopkins University School of Medicine)*

The T. brucei mitochondrion contains a proteasome-like protease related to bacterial HslVU. RNAi-mediated knockdown of TbHSLVU leads to uncontrolled kinetoplast DNA (kDNA) replication, with markedly increased levels of both maxicircles and minicircles (Li et al, 2008). We hypothesize that TbHSLVU controls the timing of kDNA synthesis by degrading a positive regulator of replication. A candidate for the protein controlling maxicircle replication is the TbPIF2 helicase (Liu et al, 2009). The regulator of minicircle replication and other substrates of TbHSLVU await discovery. To search for protease substrates, we fused a catalytically-dead mutant of TbHSLV subunit to the TAP tag. Overexpression of mutant TbHSLV led to entrapment of its partner proteins (TbHSLU1 and TbHSLU2), suggesting that the dissociation of TbHSLV and TbHslU1/HSLU2 is coupled with degradation of substrates. Among the proteins we identified following purification of TbHSLV-TAP and mass spectrometry was Tb927.10.15660, a previously uncharacterized protein. We determined that Tb927.10.15660 is a mitochondrial protein that co-localizes with kDNA. The level of Tb927.10.15660 increases following TbHSLVU RNAi, suggesting that Tb927.10.15660 is a substrate of the protease. Unexpectedly, we discovered in our proteomics that the TbHSLV subunit is phosphorylated at a unique tyrosine. To examine the role of this modification, we constructed mutants of TbHSLV with different amino acid substitutions, and overexpressed the variants in trypanosomes. Overexpression of TbHSLV with either the Y162F or Y162A substitution arrested cell growth and triggered degradation of endogenous, wild-type TbHslV with phenotypes reminiscent of TbHSLV RNAi. Cells with the phosphomimetic Y162E alteration grew normally. We propose that TbHSLV steady state level is regulated by phosphorylation, and studies are underway to characterize the mechanism of TbHSLV turnover and regulation.

2J A conserved mitochondrial outer membrane protein mediates kDNA maintenace in Trypanosoma brucei

***Schnarwiler, Felix (University Bern)**; Dewar, Caroline (Institute of Immunology & Infection Research); Schnaufer, Achim (Institute of Immunology & Infection Research); Schneider, André (University Bern)*

Kinetoplastids are defined by the unique organization of their mitochondrial DNA (kDNA). It forms a highly concatenated DNA network that is linked to the basal body of the flagellum by the tripartite attachment complex (TAC). The TAC encompasses intra and extramitochondrial filaments and a highly differentiated region of the two mitochondrial membranes. Here we identify and characterize a mitochondrial outer membrane protein of Trypanosoma brucei that is predominantly localized in the TAC. The protein is essential for growth in both life cycle stages. Immunofluorescence shows that ablation of the protein does not affect kDNA replication but abolishes the segregation of the replicated kDNA network causing rapid loss of kDNA. Besides its role in kDNA maintenance in vivo and in vitro experiments show that the protein is involved in mitochondrial protein import and that it interacts with a recently discovered protein import factor. RNAi experiments in a T. brucei cell line in which the kDNA is dispensable suggest that the essential function is linked to kDNA maintenance. Bioinformatic analysis shows that the studied outer membrane protein has beta-barrel topology and that it belongs to the mitochondrial porin family comprising VDAC, Tom40 and Mdm10. Interestingly, Mdm10 has so far only been found in yeast. Its function in protein import and mitochondrial DNA maintenance suggests that the protein in our study is the functional homologue of Mdm10. Thus, the TAC – a defining structure of kinetoplastids – contains a conserved protein which in yeast and trypanosomes performs the same function. Our study therefore provides an example that trypanosomal biology, rather than being unique, often simply represents a more extreme manifestation of a conserved biological concept.

Talk Session III: Drugs and Methods

(Chair) Michael Pollastri

04/22/2013

3A Vacuolar ATPase knockdown in T. brucei allows for kinetoplast-independent growth and resistance to the major veterinary drug isometamidium

Baker, Nicola (Lshtm)*; Barrett, Michael P. (University of Glasgow); Horn, David (Lshtm)*

Isometamidium (ISM) is a veterinary drug that accumulates in the kinetoplast (mitochondrial DNA) of trypanosomes, but our understanding of the mechanism of killing and potential mechanisms of resistance remains incomplete. We used genome-scale RNA interference (RNAi) library screening in bloodstream-form T. brucei to identify ISM resistance mechanisms. The screen linked multiple V-type ATPase subunits to ISM resistance. Knockdown of two distinct subunits led to a greater than 100-fold increase in resistance to ISM and chemical inhibition (with bafilomycin) phenocopied this effect. Localization to the lysosome was confirmed but, surprisingly, drug uptake was undiminished following knockdown. Instead, we found an unexpected link between the V-ATPase and the kinetoplast; although normally essential, kinetoplast loss (induced by acriflavine) was well-tolerated following V-ATPase knockdown. V-ATPase knockdown also rendered the cells oligomycin-resistant suggesting bypass of the mitochondrial F 0 F 1 ATPase requirement. Ethidium bromide (EBr) and pentamidine also interact with mitochondrial DNA and V-type ATPase knockdown increased resistance to EBr but had relatively little impact on resistance to pentamidine. Thus, ISM acts primarily by targeting the kinetoplast while pentamidine appears to have at least one alternative target relevant to efficacy. Our results also reveal an unexpected link between lysosomal and mitochondrial ATPases.

3B Identification of kinetoplastid deubiquitinating enzymes and their inhibitors reveal a novel target-lead pair for antiparasite drug development

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Deubiquitinating enzymes (DUBs) are proteases that remove the posttranslational modifier ubiquitin from protein substrates and thereby regulate a variety of ubiquitin-dependent processes that are vital to cellular homeostasis, including protein degradation, trafficking, DNA repair and transcription. Interfering with ubiquitin conjugation and hydrolysis is considered a promising strategy to kill aberrant cells and DUBs are currently being pursued as anticancer drug targets. Kinetoplastids also have a functional ubiquitin system and the genomes of these parasites indeed suggest the presence of DUB orthologues. Recently, a few have been described, but the identity and function of the majority of kinetoplastid DUBs remains to be unveiled. We set out to characterise all DUBs present in kinetoplastids and to explore their druggability. To this end we have developed fluorescent ubiquitin-based activity probes. The use of these probes for activity-based protein profiling revealed the presence of a multitude of DUB activities in trypanosomes and leishmania and the identity of these DUBs was confirmed by MS. Chemical genetic screening of our unique 400-membered collection of DUB inhibitors on bloodstream form T. brucei furnished highly trypanotoxic compounds (some with EC 50 <3 nM), several of which selectively target kinetoplast DUBs over mammalian DUBs. For each of the 20 cysteine protease DUBs predicted in T. brucei we have generated a stable tetracyclin-inducible RNAi line. Analysis of this set of cell lines revealed at least one DUB that appears to be essential for trypanosome survival. Our approach of combining chemical and functional genetic screening has now rendered an essential trypanosomal DUB and its inhibitor. This target-lead pair is about to enter pharmaceutical development for the treatment of Human African Trypanosomiasis.

3C New progress on the mode of action of and tolerance to miltefosine in L. infantum using metabolomics

Vincent, Isabel M. (Universite Laval)*; Weidt, Stefan (Glasgow University); Rivas, Luis (Centro de Investigaciones Biológicas (CSIC)); Burgess, Karl (Glasgow University); Ouellette, Marc (Universite Laval)*

Miltefosine is the only orally available leishmanicide and has been licensed for more than 10 years for the treatment of visceral and cutaneous leishmaniasis, even though its mode of action (MOA) is unknown. Contradictory theories as to the MOA may indicate the presence of multiple targets or may be due to the different experimental designs and analyses carried out. The three main theories for the MOA in Leishmania include alterations to the membrane lipid content, induction of apoptosis and modulation of macrophage responses. Here we perform untargeted metabolomics to elucidate the metabolic changes involved in miltefosine action. Nearly 900 metabolites were detected, 10 % of which were significantly altered after 3.75 hours. Many of the changes related to an increase in lipid breakdown leading alkane fragments and sugar release. Fragment release is synchronised with reactive oxygen species (ROS) production, which presumably causes lipid peroxidation leading to the breakdown of native phospholipids. Signs of DNA damage were also detected as were changes to the levels of some thiols and polyamines. After five hours of miltefosine treatment the cells showed depleted levels of most metabolites, which would indicate that the cells' outer membranes integrity had become compromised and internal metabolites were escaping. In miltefosine resistant cells, the drug was not internalised and the changes to the internal metabolite levels were not seen. Cells resistant to SbIII had slightly depleted levels of miltefosine uptake and a corresponding depletion in the levels of internal metabolite changes. This work will be important do inform the design of combination therapies to combat leishmaniasis, something that the research community should be prioritising in the coming years.

3D Inhibition of Trypanosoma brucei cathepsin-L increases sensitivity to lysis by human serum

Alsford, Sam (London School of Hygiene & Tropical Medicine)*; Horn, David (London School of Hygiene & Tropical Medicine)*

Human serum trypanolytic factor (TLF) is a potent anti-trypanosomal agent, with an EC50 of <0.00035%. Trypanosoma brucei takes up TLF by receptor-mediated endocytosis via the haptoglobin-haemoglobin receptor (HpHbR). Following transit to the lysosome, the lytic component of TLF, apolipoprotein-L1 (apo-L1), disrupts the lysosomal membrane, leading to parasite swelling and cellular lysis. A more detailed understanding of this process may present opportunities to develop improved therapies. We selected a genome scale T. brucei RNAi library with human serum and identified several factors that normally sensitise trypanosomes to TLF, including HpHbR, the lysosomal protein p67 and ‘inhibitor of cysteine peptidase’ (ICP). Ablation of ICP increased resistance to human serum. Notably, this phenotype was wholly reversed by treatment with FMK024, a cathepsin (Cat)-B/L inhibitor, or specific RNAi depletion of lysosomal Cat-L. Thus, enzymatic, chemical and RNAi-based inhibition of Cat-L all demonstrate that this protease can afford some protection against TLF, likely through proteolytic destruction of apo-L1 in the lysosome

3E The dynamic nature of experimental chronic Chagas disease revealed by highly sensitive in vivo imaging

***Lewis, Michael**; Burrell-Saward, Hollie; Fortes Francisco, Amanda; Kelly, John; McLatchie, Alex; Miles, Michael; Taylor, Martin*

Chronic Trypanosoma cruzi infections either remain asymptomatic or result in cardiac and/or digestive pathologies. During the chronic stage of Chagas disease parasites are thought to be sequestered in diverse tissues, but links between parasite tissue tropism and disease pathogenesis have been poorly defined. To address this we developed a real-time bioluminescence imaging infection model based on a transgenic T. cruzi cell line, constitutively expressing the 'red-shifted' firefly luciferase variant Ppy RE9. Luciferase expression levels were tightly correlated with parasite number and significant reduction of bioluminescence was observed after oral treatment of acute and chronic infections with benznidazole. The in vivo limit of detection was <1000 parasites per animal and therefore vastly more sensitive than peripheral blood parasitaemia counts. In a model of acute fulminating disease in an immunuocompromised host (SCID mouse) ex vivo imaging of tissues showed the highest parasite burden to be harboured by visceral fat depots. Parasite burdens in immunocompetent BALB/c mice peaked at 14 d.p.i. and could be visualised for >250 days. Chronic parasite foci were highly dynamic with a high degree of spatial variation and parasite burden intensity fluctuating over 2 logs of magnitude. Ex vivo imaging revealed that the large intestine was the primary site of chronic (153 dpi) parasite persistence with 5 to 12-fold higher burdens compared to the heart. Nevertheless, mice developed myocarditis and progressive heart fibrosis. These data imply that chagasic cardiac pathology may not result exclusively from local parasitism and the specific immune response directed against it.

3F Expression GPIomics of the Mammal-Dwelling Stages of Trypanosoma cruzi

***Lopes, Felipe G.**; Aguilar, Clemente; C. Almeida, Igor; F. Marques, Alexandre; S. Nakayasu, Ernesto*

Glycosylphosphatidylinositol (GPI)-anchoring is a protein post-translational modification ubiquitously found in eukaryotes. There is a growing body of evidence showing that protein-free GPIs (or glycoinositolphospholipids, GIPLs) and GPI-anchored proteins (GPI-APs) are involved in host-protozoan interaction processes, such as host-cell adhesion and invasion, and pathogenesis. Here, we used a highly sensitive and objective approach that employs liquid chromatography-tandem mass spectrometry (LC-MSn) for the analysis of the GPIome (GPIomics) of the mammal-dwelling trypomastigote and amastigote stages of Trypanosoma cruzi, the causative agent of Chagas disease. This approach allows for the structural characterization of both the lipid and the glycan moieties of GPIs. We have so far identified over 150 GIPL species from these two parasite forms, most of which had not been described in the literature. In contrast to epimastigote-derived GIPLs (eGIPLs), trypomastigote-derived GIPLs (tGIPLs) tend to have longer glycan moieties, many of which contain pentose (xylose) residues. On the other hand, intracellular amastigote-derived GIPLs (aGIPLs) contain sugar moieties that tend to be somewhat less diverse, although on average they are also larger than the ones found in eGIPLs. The lipid moieties of tGIPLs are composed mainly of ceramides, typically with longer fatty acid chains than those of eGIPLs. Conversely, GIPLs tend to have mostly 1-O-alkyl-2-O-acyl-glycerolipid moieties. These traits are probably related to the specific needs of each life-stage. We are currently conducting the proteomic and GPIomic analyses of the GPI-APs derived from these two mammal-dwelling life-cycle stages, which will help us further understand possible structure-function correlations of GPIs and their role in the chronic infection.

3G Quantitative phosphoproteomic analysis of procyclic and bloodstream form Trypanosoma brucei reveals potential roles for phosphorylation in adaptation

Urbaniak, Michael*; Ferguson, Michael A.J.; Guther, M. Luca S.; Martin, David M. A.*

Trypanosoma brucei has a complex digenetic lifecycle between a mammalian host and an insect vector, and adaption of its proteome between lifecycle stages is essential to its survival and virulence. Here, we report the global quantitative proteomic and phosphoproteomic analysis of bloodstream and procyclic form T. brucei using stable isotope labelling by amino acids in culture (SILAC) of each lifecycle stage. We were able to identify 5,478 proteins and quantify SILAC ratios for 3,763 proteins, with >10% of proteins differentially regulated >5-fold between lifecycle stages, including those involved in the parasite surface coat, and in mitochondrial and glycosomal energy metabolism. We observe usually strong correlation (0.85) between changes in protein levels and the change in mRNA levels reported in previous transcriptomic studies, supporting the hypothesis that the post-transcriptional regulation of mRNA level is a major component in the regulation of gene expression in T. brucei. Phosphopeptide enrichment by SCX and TiO2 lead to the identification of 10,096 phosphorylation sites (>90% new) on 2,551 proteins, and quantified the ratios of 8,275 phosphorylation sites between the two lifecycle stages. Widespread differential protein phosphorylation occurs between bloodstream and procyclic from trypanosomes, with significant intra-protein differential phosphorylation. Gene enrichment analysis of phosphorylated proteins identified GO terms relating to the flagella, protein kinase activity, and the regulation of gene expression. Despite a lack of dedicated tyrosine kinases, phosphotyrosine residues were 3-4 fold over-represented amongst site changing >10-fold between the two lifecycle stages. The majority of the T. brucei kinome was phosphorylated, with evidence that MAPK pathways are functional in both lifecycle stages. The extensive phosphorylation of RNA binding proteins observed may be relevant to the control of mRNA stability.

3H Use of the HaloTag to modulate protein level in Trypanosoma cruzi and Trypanosoma brucei.

***Jones, Deuan**; De Rycker, Manu; Fairlamb, Alan; Patterson, Stephen*

A wide variety of drug targets have been assessed in Trypanosoma brucei fuelling successful drug discovery campaigns in both academic and industrial settings. By contrast there is a scarcity of validated drug targets in Trypanosoma cruzi in part due to a paucity of genetic tools for assessing essentiality. RNAi is not possible in T. cruzi and no conditional expression system is in wide use. We have used the HaloTag degradation system previously used in higher organisms [1] to target luciferase for destruction in T. cruzi. This system uses small ‘tagging’ molecules which irreversibly bind to a HaloTag fusion protein. The tagged protein displays a hydrophobic moiety, which causes it to be degraded via the proteasome depleting the target much more rapidly than expression-targeting approaches. Using a cytotoxicity assay optimised for epimastigotes together with a high-content screen for intracellular amastigotes, we have synthesised and identified suitable tagging compounds and established optimum conditions to induce degradation of luciferase in T. cruzi . We have also assessed the system in T. brucei. Whilst generation of conditional null mutants is relatively straightforward in these parasites, the HaloTag system can be used for target assessment in vivo. Our approach could provide a more realistic surrogate for small-molecule mediated inhibition of a target, where complete inhibition is an inadvisable goal to set during target assessment. A similar strategy for Leishmania spp is under development. [1] Neklesa TK, Tae HS, Schneekloth AR, et al. Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. Nat Chem Biol 2011;7:538-43.

3l Identification of the mRNA-fate modulators through a high-throughput tethering assay

***Erben, Esteban D.**; Clayton, Christine; Fadda, Abeer; H.V. e Vieira, Carlos*

The regulation of mRNA decay and translation is central to post-transcriptional regulation of gene expression in trypanosomatids. A complete understanding of these dynamic processes will be possible only if we identify all the trans-acting factors involved. Genome-wide screens have proven powerful in associating gene products with certain phenotypes or signal transduction pathways, and thus are valuable tools to ascribe gene function. Methods for high-efficiency transfection have made it possible to perform systematic genome-wide functional screens. We intend to discover genes that regulate mRNA and protein abundance. Here, we report on the application of an overexpression screen to identify the full set of mRNA-fate regulators. Specifically, we adapted a high-throughput tethering assay which relies on stabilization of a reporter. We have used the screen to uncover genes that, when overexpressed, increase the expression of the reporter gene, either by mRNA stabilization or enhancement of translation efficiency. Our findings reiterate the role that well-characterized proteins play in the regulation of the mRNA fate, and suggest new roles for previously uncharacterized and ‘‘hypothetical’’ genes. In addition, our screen provides a fine structure mapping of the hit candidates’ functional domains. We are currently verifying the functions of several novel candidate regulators. In addition, we are extending the screen to look for proteins that destabilize mRNAs or inhibit translation. We hope that our work will help us to decipher the codes that control gene expression, while also providing a resource to further understand the function of the whole African trypanosome genome.

Talk Session IV: RNA Metabolism
(Chair) Keith Matthews

04/23/2013

4A Interactions of LEISHMANIA RNA Virus 1 (LRV1) with the RNA interference pathway

Beverley, Stephen M. (Washington University School of Medicine)*; Lye, Lon-Fye (Washington University School of Medicine); Akopyants, Natalia (Washington University School of Medicine); Shaik, Jahangheer (Washington University School of Medicine); Brettman, Erin (Washington University School of Medicine); Kuhlmann, F. Matthew (Washington University School of Medicine); Zangger, Haroun (University of Lausanne); Ronet, Catherine (University of Lausanne); Fasel, Nicolas (University of Lausanne)*

The dsRNA Leishmania virus 1 (LRV1) persistently infects species within the subgenus Viannia. L. guyanensis LRV1 has been associated with hypervirulence and increased metastasis, the latter being a hallmark of the more severe forms of leishmaniasis (Ives et al. Science 2011). Intriguingly, while many Leishmania have lost the RNA interference (RNAi) pathway, Viannia have retained it (Lye et al PLoS Pathogens 2010). RNAi is a useful tool playing key roles with viruses, and we are studying its roles in Leishmania. We surveyed L. braziliensis strains for the presence of LRV1 (Zangger et al PLoS NTD 2013), and selected three for complete viral genome and small RNA sequencing. The Lbr LRV1 genomes resembled Lguy LRV1, including the +1 capsid/RNA-dependent RNA polymerase frame. Lbr LRV1s showed extensive nucleotide divergence (up to 18%), approaching that of the Lguy-Lbr LRV1s (22%). Lbr and Lguy LRV1-infected parasites showed high levels of viral siRNAs, from 0.4-1.5% of total siRNAs, whose structure and size mirrored normal cellular siRNAs (Atayde et al Mol Micro 2013). LRV1 siRNAs mapped broadly across both strands of the LRV1 genome, albeit with several ‘hot spots’. Viral siRNA levels were robust, comparable to those arising from a highly active luciferase stem-loop transgene (LUC-StL) which extinguished luciferase reporter expression. We then explored the ability of an LRV1 stem-loop transgene to target LRV1. Analysis of LRV1 capsid levels by FACS and viral RNA by RT-PCR showed dramatic reductions in LRV1. This has important practical applications in the generation of isogenic lines lacking LRV1s. An unanswered question is how LRV1, which resides and replicates in the cytosol, is able to survive recognition and attack by the endogenous RNAi pathway, yet can fall prey to attack when targeted by the 'exogenous' nuclear LRV stem-loop transgene.

4B Leishmania aethiopica field isolates with an immunogenic Leishmania RNA virus

Zangger, Haroun (University of Lausanne)*; Desponds, Chantal (University of Lausanne); Lye, Lon-Fye (Washington University School of Medicine); Hailu, Asrat (Addis Ababa University); Beverley, Stephen (Washington University School of Medicine); Fasel, Nicolas (University of Lausanne)*

Leishmania RNA virus (LRV) is a double-stranded RNA virus that has been detected in Leishmania (Viannia) braziliensis and guyanensis species, which can cause not only cutaneous but also mucocutaneous (MCL) and disseminated (DCL) leishmaniasis. This virus is composed of a capsid protein, a RNA dependent RNA polymerase (RdRp) and of a dsRNA genome of 5.3kb. In a mouse model, we showed that the viral dsRNA genome in L. guyanensis parasites is recognized by the host endosomal Toll-like receptor 3 (TLR3) and induced proinflammatory cytokines and chemokines. These TLR3-dependent immune responses render mice more susceptible to infection, and the animals develop an increased footpad swelling and parasitemia. MCL and DCL have also been described in other parts of the world, e.g. in Ethiopia. We detected naturally occurring LRV within L. aethiopica parasites isolated from patients. Three LRV genomes were sequenced from independent isolates confirming that LRV in L. aethiopica (Lae -LRV) belongs to the same Totiviridae family of LRVs found in south american species and present in a single isolate of L. major . Lae -LRV genomic organization is similar but not identical to the other LRVs. While LRV1 from L. braziliensis and guyanensis displays a +1 difference in the capsid/RdRp reading frame, and LRV2 of L. major capsid/RdRp polypeptides are encoded in the same frame, Lae -LRV genome displays a -1 difference in the capsid/RdRp reading frame. Similarly to L. guyanensis , the presence of LRV in L. aethiopica induced a TLR-3 inflammatory response in infected bone marrow macrophages. The presence of LRV and its detection could be a crucial step towards the development of new diagnostics and treatments for L. aethiopica infected patients.

4C The Trypanosoma brucei siRNA exonuclease TbRIF4 has a second role in the maintenance of TbAGO1 protein levels

Barnes, Rebecca (Yale University)*; Ramey-Butler, Kiantra (Yale University); Kolev, Nikolay (Yale University); Shi, Huafang (Yale University); Tschudi, Christian (Yale University); Ullu, Elisabetta (Yale University)*

A central step in the RNA interference (RNAi) pathway in Trypanosoma brucei is carried out by TbRIF4, which processes duplex small interfering RNAs (siRNAs) to single-stranded form, and loads them into the Argonaute Slicer (TbAGO1) to guide destruction of homologous transcripts. We have identified a second role for RIF4 in maintaining AGO1 protein levels: in the absence of RIF4 AGO1 levels decrease by ~20 fold. This role is specific to RIF4 as genetic ablation of each of the other three RNAi pathway genes only decreases AGO1 levels by ~30%. AGO1 is an abundant (~60,000 molecules/cell), mostly cytoplasmic, protein and its level strictly correlates with the efficiency of RNAi. We have previously shown that AGO1 and RIF4 interact in vivo and have determined by mass spectroscopy that no other abundant proteins are in the complex. Initial investigations of possible reasons for the reduction in AGO1 levels revealed that that there is only a slight reduction in AGO1 mRNA and no difference in AGO1 half-life between wild-type and rif4-/- cells, and that AGO1 is not proteasomally or lysosomally degraded in the absence of RIF4. In vivo UTR swapping experiments demonstrated that the AGO1 ORF, but not its UTRs, are required for AGO1 accumulation, and expression of AGO1 in an in vitro transcription-translation system required either co-translation of RIF4 or addition of the chemical chaperone DMSO. In vitro siRNA loading into AGO1 is not necessary for its accumulation and RIF4 exonuclease mutants can recover AGO1 levels in vitro and in vivo. Our results indicate that AGO1 protein levels are maintained by a translational control mechanism that is dependent on RIF4. We are currently investigating which step in translation is affected by RIF4.

4D Regulation of RNA metabolism by ARE-binding proteins in Trypanosoma brucei

Lu, Zhiquan (McGill University); *Najafabadi, Hamed Shateri (University of Toronto); Mehta, Vaibhav (McGill University); Gazestani, Vahid Hajihoseini (McGill University); Adoue, Veronique (McGill University); Salavati, Reza (McGill University)*

In trypanosomes, gene expression is regulated mainly through differential mRNA decay or other post-transcriptional mechanisms, mediated by a poorly characterized network of cis- and trans-acting elements that encompasses interaction of RNA-binding proteins (RBPs) with particular sequence elements of their target mRNAs. Our computational studies have identified highly conserved adenosine-uridine (AU)-rich elements (AREs) in the 3' untranslated regions (UTRs) of a large number of T. brucei mRNAs. These ARE-containing transcripts are up-regulated in the stationary-phase of in vitro-cultured procyclic form cells and down-regulated in stumpy and slender bloodstream form cells, suggesting a role for AREs in the regulation of parasite differentiation. Through sequence analysis of T. brucei RBPs, we have identified three potential remote homologs of ELAV (Embryonic Lethal, Abnormal Vision) proteins. In higher eukaryotes, proteins of the ELAV-like family regulate gene expression by stabilizing or promoting degradation of ARE-containing transcripts, suggesting a similar role of these proteins in regulating trypanosomatid ARE-containing mRNAs. Using in vitro electrophoretic mobility shift assays (EMSA) and RNA immunoprecipitation followed by deep sequencing (RIP-Seq), we confirmed that the T. brucei ELAV-like proteins bind specifically to AREs. Furthermore, microarray analysis of over-expression and RNAi knockdown cell lines suggests that the three identified ELAV-like proteins specifically regulate expression of ARE-containing transcripts. In addition, tandem affinity purification (TAP) followed by mass spectrometry revealed association of ELAV-like proteins with a number of other RBPs that may be involved in ARE-mediated mRNA turnover. Together, these results suggest a widespread role of ELAV-like proteins in genome-wide regulation of trypanosomatid mRNAs, and warrant further studies to examine the role of these proteins in T. brucei life cycle progression and infection.

4E Genome-wide RNA-binding analysis of the trypanosome U1 snRNP-specific proteins U1C and U1-70K provides evidence for a spliceosomal network linking cis- and trans-splicing

Preußer, Christian (Justus Liebig University of Giessen)*; Roßbach, Oliver; Hung, Lee-Hsueh; Li, Dan; Bindereif, Albrecht (Justus Liebig University of Giessen)*

Maturation of polycistronic pre-mRNAs in trypanosomes require processing of the primary transcript by coupled trans-splicing and polyadenylation, to generate proper 5‘ and 3’ ends of the mRNA, respectively. Splicing is catalyzed by the spliceosome, which contains the small ribonucleoprotein particles (snRNPs) U1, U2, U4/U6, and U5, and in addition the spliced leader (SL) RNP. During trans-splicing, a 39-nucleotide mini-exon, which is derived from the SL RNA, is attached to the 5’ end of each protein-coding sequence. In contrast, the U1 snRNP is required for cis-splicing of the two known intron-containing genes in Trypanosoma brucei. To gain more insights into putative new functions of the U1 snRNP, we adapted the genome-wide crosslinking-immunoprecipitation approach (iCLIP-Seq) to the trypanosome system, focussing on the U1 snRNP-specific proteins U1C and U1-70K. Surprisingly, both proteins interact not only with the U1, but also with U6 and SL RNAs, suggesting a direct link between the cis- and trans-splicing machinery. In addition, mapping of crosslinks to the cis-spliced PAP [poly(A) polymerase] pre-mRNA indicate an active role of the U1 snRNP proteins in 5’ splice site recognition. Our results imply, that the U1 snRNP may represent an evolutionary link between cis- and trans-splicing, playing a dual role in 5’ splice site recognition on the SL RNP, which needs to activate its own 5’ splice site during trans-splicing, as well as on the cis-spliced pre-mRNAs. Taken together we could demonstrate that the iCLIP approach promises to become a valuable tool in mapping stable and transient RNA-protein contacts in trypanosomes.

4F Architecture of the RNA Editing Holoenzyme

Afasizhev, Ruslan (Boston University)*; Huang, Lan (University of California-Irvine); Afasizheva, Inna (Boston University)*

Mitochondrial RNA editing is catalyzed by a well-characterized RNA editing core complex composed of ~15 proteins. However, the nature of axillary factors required for the editing process and its connections with other RNA processing steps and translation remain poorly understood. Building on our earlier discovery of two RNA binding proteins, GRBC1 and 2, which are essential for guide RNA stability we set out to determine a complete proteome of macromolecular complexes involved in gRNA biogenesis, pre- and post-editing mRNA processing, and translation. Iterative building of protein interaction networks and functional analyses of individual subunits demonstrated that a tetramer composed of two GRBC1-2 dimers represents the gRNA binding interface of a multi-protein platform termed gRNA binding complex (GRBC). This platform also binds pre-edited and edited mRNAs and interacts with core editing, polyadenylation and REH2 helicase complexes via specific modules. Overall, we found that RNA substrate-mediated assembly of enzymatic editing core and gRNA binding complexes represents the RNA editing holoenzyme. Furthermore, this superassembly is associated with large ribosomal subunit by means of direct protein contacts with GRBC. The majority of nearly 20 GRBC subunits are essential for cell viability with their knockdowns often causing transcript-specific editing defects. Distribution of pre-edited and edited mRNA forms among complexes demonstrated that the fully-edited mRNA is likely to be actively disengaged from large ribosomal subunit-bound GRBC. This event apparently takes place prior to addition of the 200-300 nt-long 3' A/U-tail by KPAP1 poly(A) polymerase and RET1 TUTase, which commits mRNA for small ribosomal subunit binding and translation. In summary, our findings indicate that gRNA biogenesis, pre-editing, editing and post-editing mRNA modifications are tightly coupled with mitochondrial translation.

4G Mechanism of spliced leader RNA silencing (SLS) and factors involved in the controlling the of SLS-induced programmed cell death

***Michaeli, Shulamit (Bar-Ilan University)**; Hope, Ronen (Bar-Ialn University); Ben-Mayor, Efrat (Bar-Ilan University); Biswas, Dipul (Bar-Ilan University); Voloshin, Konstatin (Bar-Ilan University); Gunzl, Arthur (Bar-Ilan University)*

Trypanosomes appear to lack conventional mechanisms of transcription regulation, including factors that induce the unfolded protein response (UPR). However, trypanosomes do possess a stress response mechanism, the spliced leader RNA (SL) RNA) silencing (SLS) pathway. SLS is induced under prolonged ER stress or when translocation to the ER is perturbed upon RNAi silencing of factors involved in protein translocation across the ER membrane such as SRP receptor, SEC63 or SEC61 - the translocation channel (Lustig et al., 2007, EMBO Rep). SLS leads to shut-off of SL RNA gene transcription by perturbing the binding tSNAP42 to the SL RNA promoter. Induction of SLS leads to programmed cell death (PCD) (Goldshmidt et al., 2010, PLoS Pathogens). SLS serves as is a unique death pathway of trypanosomes, replacing the conventional caspase-mediated PCD present in higher eukaryotes (Michaeli, 2012, Parasite and Vectors). To investigate the shut-off mechanism of SL RNA gene transcription during SLS, the SL RNA transcription factor complex was purified under SLS by using tSNAP42, tSNAP26 or TRF4 (TBP) PTP-tagged proteins. Mass-spectrometric analysis indicated that SLS induced phosphorylation of TBP on serine 35. A PERK-like kinase that we termed PK-3 was co-purified with the transcription factor complex under SLS. During SLS PK-3 moves from the surface of the ER to the nucleus and phosphorylates TBP. Silencing of this kinase, abolished SLS and slowed –down the PCD process, suggesting that SLS induction involves a phosphorylation cascade. To further investigate factors essential for SLS induction we examined changes in proteome using iTRAQ. We focused on two proteases that were up-regulated in SLS. Rhomboid-like protease moves from the mitochondrion to the ER during SLS and is essential for protein translocation across the ER. Silencing the expression of calpain-like protease abolished SLS, suggesting that this protein is essential for PCD induction under SLS.

4H Genome-wide analysis of translation in Trypanosoma brucei using ribosome profiling

Jensen, Bryan C. (Seattle Biomedical Research Institute)*; Ramasamy, Gowthaman (Seattle Biomedical Research Institute); Haydock, Andrew (Seattle Biomedical Research Institute); Ingolia, Nicholas (Carnegie Instituition); Myler, Peter (Seattle Biomedical Research Institute); Parsons, Marilyn (Seattle Biomedical Research Institute)*

While RNA-seq excels at quantification of the relative levels of all mRNAs, these levels do not necessarily reflect the relative abundance of the corresponding proteins. Conversely, even the latest generation mass spectrometers are unable to give a comprehensive picture of protein abundance. To bridge the gap between these two methods, Ingolia et al (Science 2009) developed ribosome profiling, an approach that comprehensively determines the relative translation of all mRNAs. This method involves treatment of cell lysates with RNase to digest the mRNA except for the ~28 nucleotide region protected by the ribosome. The protected mRNA fragments are purified and identified by RNA-seq. Comparison of ribosome-protected read counts with those obtained from total mRNA provides a global picture of the relative translation efficiency for all mRNAs. This technique also allows for proper identification of canonical and non-canonical start, upstream reading frames, and previously unidentified protein coding sequences. Our data generated from Trypanosoma brucei cultured bloodstream and procyclic forms provides a comprehensive picture of the total translational capacity of both life cycle stages. Hundreds of genes show different translation efficiencies between the two stages. For many genes, translational control appears to be the dominant mechanism of stage-regulated expression, although it is frequently combined with changes in mRNA abundance. Examples of the former include Tb927.10.13540 and Tb11.01.3915 (which encode the RNA binding proteins RBP12 and RPB5, respectively); while the latter include Tb927.6.440 and Tb927.1.4100 (which encode haptoglobin-hemoglobin receptor and cytochrome oxidase IV, respectively). In addition, we identified tens to hundreds of unannotated protein-coding genes, some of which are developmentally regulated. This technique is currently being expanded to additional life cycle stages and Leishmania.

4l High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome

hashem, yaser (Columbia University); *Des Georges, Amedee; Fu, Jie; Buss, Sarah N.; Jossinet, Fabrice; Jobe, Amy; Zhang, Qin; Liao, Hstau Y.; Grassucci, Robert A.; Bajaj, Chandrajit; Westhof, Eric; Madison-antenucci, Susan; Frank, Joachim*

Ribosomes, the protein factories of living cells, translate genetic information carried by messenger RNAs into proteins, and are thus involved in virtually all aspects of cellular development and maintenance. The few available structures of the eukaryotic ribosome reveal that it is more complex than its prokaryotic counterpartowing mainly to the presence of eukaryote-specific ribosomal pro- teins and additional ribosomal RNA insertions, called expansion segments. The structures also differ among species, partly in the size and arrangement of these expansion segments. Such differences are extreme in kinetoplastids. Here we present a high-resolution (~5A) cryo-electron microscopy structure of the ribosome of Trypanosoma brucei, the parasite that is transmitted by the tsetse fly and that causes African sleeping sickness. The atomic model reveals the unique features of this ribosome, characterized mainly by the presence of unusually large expansion segments and ribosomal protein extensions leading to the formation of four additional inter-subunit bridges. We also find additional rRNA insertions, including one large rRNA domain that is not found in other eukaryotes. Furthermore, the structure reveals the five cleavage sites of the kinetoplastid large ribosomal subunit rRNA chain, which is known to be cleaved uniquely into six pieces, and suggests that the cleavage is important for the maintenance of the T. brucei ribosome in the observed structure. We discuss several possible implications of the large rRNA expansion segments for the translation regulation process and we show a possible link between the protein translation initiation process and expansion segments 6 and 7 on the small ribosomal subunit. The structure could serve as a basis for future experiments aimed at understanding the functional importance of these kinetoplastid-specific ribosomal features in protein translation regulation, an essential step towards finding effective and safe kinetoplastid-specific drugs. Reference: Hashem Y. et al., Nature 2013 (doi:10.1038/nature11872).

Talk Session V: Biochemistry
(Chair) Nicola Carter

04/24/2013

5A Lipid metabolism in Trypanosoma brucei and other kinetoplastids: so much more than just membranes!

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The membranes of the rapidly dividing Trypanosoma brucei contain a complex array of phospholipids that are de novo synthesized from precursors obtained either directly from the host, or as catabolised endocytosed lipids. This includes phosphatidylcholine and sphingomyelin which contribute ~65% to the parasites membranes. Our current understanding of choline metabolism and the various roles it plays in the different life-cycles will be presented. The lack of transport and lack of de novo synthesis of choline in bloodstream form T. brucei will be highlighted, along with the current possibilities for therapeutic intervention in this area. This research is supported in part by the Wellcome Trust and SULSA.

5B Leishmania Rely on Ethanolamine for Phospholipid Synthesis

Pawlowic, Mattie C. (Texas Tech University)*; Hsu, Fong-Fu (Washington University School of Medicine); Zhang, Kai (Texas Tech University)*

For Leishmania promastigotes, ethanolamine (EtN) is an essential nutrient synthesized via sphingoid base metabolism. EtN is an important precursor of phospholipids, the most abundant of which are phosphatidylethanolamine (PtE) and phosphatidylchloline (PtC). Like most organisms, Leishmania synthesize PtE and PtC through the Kennedy pathway, which consists of two parallel branches converting EtN to PtE and choline to PtC. While Leishmania PtC is heterogeneous in its acyl chain composition, Leishmania PtE is almost exclusively 1-alkenyl-2-acyl-PtE (PLE), a plasmalogen phospholipid. Genetic knockout experiments demonstrate that PLE-null parasites have defects in the synthesis and trafficking of GPI-anchored virulence factors. These mutants also show reduced infectivity in mice. However, their ability to proliferate as procyclics and to differentiate into metacyclics remains unaltered. These results suggest that the essential role of EtN extends beyond PLE synthesis. Importantly, unlike T. brucei , Leishmania incorporate EtN into both PtE and PtC. Preliminary investigation of Leishmania homologs from both braches of the Kennedy pathway indicate that while the EtN-branch enzymes are essential for parasite survival, the choline-branch enzymes may not be required for PtC synthesis. This suggests that EtN is essential in Leishmania because it is the preferred precursor of PtC synthesis. We hypothesize that the high demand for PtC (approximately 40% of total cellular lipids) drives the de novo synthesis of EtN despite the risk of generating toxic intermediates from sphingoid base metabolism.

5C Trypanosomatid deoxyhypusine synthase requires heterotetramer formation with a catalytically dead paralog which serves as an activator domain.

Nguyen, Suong (University of Texas Southwestern Medical Center)*; Jones, Deuan (University of Dundee); Wyllie, Susan (University of Dundee); Fairlamb, Alan (University of Dundee); Phillips, Margaret (University of Texas Southwestern Medical Center)*

The initiation factor 5A in archaea and eukaryotes (aIF5A and eIF5A, respectively) undergoes a unique post-translational modification termed hypusination. Deoxyhypusine synthase (DHS) catalyzes the first step of the hypusination in which the aminobutyl group of spermidine is transferred onto an eIF5A lysine to form eIF5A-deoxyhypusine. In a second step catalyzed by deoxyhypusine hydroxylase, the deoxyhypusine is converted to hypusine (Ne-(4-amino-2-hydroxybutyl)-lysine). Trypanosomatids encode two DHS paralogs; only one contains the necessary catalytic lysine (DHSc, Tb927.10.2750) and the other is predicted to be catalytically inactive (DHSp, Tb927.1.870). Using a conditional knockout approach, we observed that both genes are essential for growth of cultured bloodform T. brucei and for infectivity in mice. We generated cells expressing epitope tagged DHSc and DHSp and demonstrated that they interact in vivo by co-immunoprecipitation. To dissect the enzymatic activity of DHS, we cloned and expressed both paralogs in Escherichia coli and as predicted, only DHSc had measurable activity. However, we found that DHSc:DHSp form an active heterotetramer that is 3000-fold more active than DHSc alone. This echoes the regulatory mechanism we discovered for trypanosomatid S-adenosylmethionine decarboxylase in which heterodimer formation with a catalytically dead paralog (a prozyme) leads to a 1000-fold increase in activity. Remarkably these data show that this mechanism has evolved twice and uniquely in the trypanosomatid polyamine metabolism pathway. Our results suggest that further examples of catalytically dead paralogs acting as regulatory subunits are likely yet to be discovered and the prevalence of this mechanism in the parasite may reflect the lack of transcriptional control mechanisms for gene expression.

5D Intracellular amastigote stages of Leishmania mexicana exhibit a limited capacity to utilize carbon sources other than sugars.

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Leishmania amastigotes proliferate within macrophage phagolysosomes, perpetuating disease in the mammalian host. As one of the major degradative compartments, the phagolysosome is thought to be a nutritionally complex niche that the amastigote exploits to sustain its multiple auxotrophic requirements. Previous studies have suggested that, in contrast to extracellular (insect) promastigotes, amastigotes switch to using carbon sources other than sugars such as amino acids and fatty acids within this complex environment. To further investigate carbon metabolism in Leishmania parasites, we've utilized a new comprehensive stable isotope resolved metabolomics approach to delineate both the relative contribution and fate of different carbon sources in L. mexicana promastigotes and amastigotes (axenic and lesion-derived). Firstly, using this approach we show that many metabolic fluxes are strongly repressed in both axenic and lesion-derived amastigotes, as compared to promastigotes, indicating that they enter a metabolically quiescent state. Secondly, we find that amastigotes retain the capacity to catabolize glucose via previously defined pathways (glycolysis, pentose phosphate pathway, succinate fermentation) with negligible overflow metabolism suggesting a more efficient metabolic state. Thirdly, we find that amastigotes minimally utilize amino acids but demonstrate increased catabolism of fatty acids. Specifically, acetyl-CoA, generated by fatty acid b-oxidation, is used in glutamate/glutamine biosynthesis rather then being fully oxidized by the TCA cycle. Using inhibitors, we confirm that catabolism of glucose and fatty acids, via the limited TCA cycle, is essential for intracellular growth. These findings highlight key pathways of carbon metabolism that are essential for amastigote growth and survival and are consistent with previous genetic studies.

5E Cytosolic NADPH homeostasis in glucose-starved procyclic Trypanosoma brucei relies on malic enzyme and gluconeogenic flux

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Most organisms depend on NADPH production to feed essential biosyntheses and their oxidative stress defense system. Protozoan parasites like Trypanosoma brucei adapt to different host environments, carbon sources and oxidative stresses during their life cycle. The developmental stages in the tsetse insect vector rely on proline as carbon source, although they prefer glucose when grown in rich culture media. An unanticipated flexibility was found in the use of two NADPH synthesis pathways in the cytosol of the procyclic stage, the pentose phosphate pathway (PPP) and the cytosolic malic enzyme (MEc). Targeting those pathways by reverse genetics and a chemical inhibitor we could show full redundancy in oxidative stress management. Blocking both pathways results in ~10-fold increased susceptibility to H2O2 stress and lethality. In addition, we propose a "transhydrogenase-like" cycle that can support ATP-neutral transfer of electrons from glycosomal NADH to cytosolic or mitochondrial NADPH. This could be crucial during peak demands of NADPH, e.g. when facing oxidative stress from the host environment. Surprisingly, the same pathway redundancy was also observed in glucose-depleted conditions where the PPP was thought to be essentially inactive. This suggested an alternative source of G6P, which we show to be gluconeogenesis-derived. Genetic evidence from targeting a gene essential for gluconeogenesis, encoding glucose 6-phosphate isomerase (PGI), and metabolomic evidence from [U-13C]-proline labeling will be presented. This is the first experimental evidence for gluconeogenic flux in T. brucei. Gluconeogenesis supports NADPH supply and may also be important for nucleotide and glycoconjugate syntheses in the insect host and thus for transmission. Discovery of alternative and redundant metabolic pathways only active in defined developmental stages or nutrient conditions is ongoing.

5F A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in *Leishmania donovani*.

Martin, Jessica L.; *Boitz, Jan; Carter, Nicola; Cassera, Maria Belan; Fulwiler, Audrey; Ullman, Buddy; Yates, Phillip*

The salvage of purines by Leishmania is an obligatory process that impacts both cell viability and growth. Our previous studies demonstrated that purine starvation provokes significant morphological and metabolic changes, including the upregulation of permeases and salvage enzymes involved in purine acquisition and interconversion. To understand how Leishmania sense and adapt to changes in their purine environment, we have exploited purine pathway mutants. While wild type parasites are able to transport and convert any single purine nucleobase or nucleoside to fulfill their adenylate and guanylate nucleotide requirement, these purine pathway mutants have restricted purine requirements for growth. Thus, these mutants can be maintained in high levels of an extracellular purine that is non-permissive for sustained parasite replication. By culturing these purine pathway mutants in purines permissive or non-permissive for growth and subsequently monitoring for the upregulation of specific purine transporters and salvage enzymes — hallmarks for the adaptive response to purine starvation — we have determined that the adaptation to purine starvation arises from the surveillance of intracellular purine pools rather than from the direct sensing of the extracellular purine environment. Furthermore, our data suggest that the response to purine starvation is more profound when perturbations within the adenylate nucleotide pool are elicited rather than within the guanylate nucleotide pool, suggesting that adenylate nucleotide imbalances may be a primary trigger for the response to purine stress. Using a targeted metabolomic approach for profiling purine metabolites, we found that changes in intracellular purine pools occur within 2 h of purine removal from the media, and significantly, that at 24 h extreme changes in the levels of AMP, ADP, and ATP are evident.

5G GMPS: The "Achilles's Heel" of Purine Metabolism in *Leishmania donovani*

Boitz, Jan M. (Oregon Health & Science University)*; Fulwiler, Audrey (Oregon Health & Science University); Ullman, Buddy (Oregon Health & Science University)*

An intricate genetic dissection of the purine salvage pathway of Leishmania donovani has revealed that this parasite can fulfill its nutritional needs by funneling any naturally occurring host purine into hypoxanthine, guanine, or xanthine. These purines are then incorporated into the parasite nucleotide pool through either hypoxanthine-guanine phosphoribosyltransferase (HGPRT) or xanthine phosphoribosyltransferase (XPRT). The phenotypic characterization of a conditionally lethal ∆hgprt/∆xprt line, has verified that either HGPRT or XPRT is both necessary and sufficient for purine salvage by L. donovani and implied that downstream nucleotide interconversion enzymes including inosine monophosphate dehydrogenase (IMPDH), adenylosuccinate synthetase (ADSS), adenylosuccinate lyase (ASL), GMP reductase (GMPR) and GMP synthase (GMPS) could be essential to the parasite. Using rational selective strategies, ∆impdh, ∆gmpr, ∆adss and ∆asl null mutants were readily generated under permissive growth conditions. However, despite numerous attempts and intricate pharmacological and nutritional manipulations of the selection medium, we were unable to isolate a ∆gmps mutant implying that a ∆gmps mutation is lethal. We hypothesized that a ∆gmps lesion might be lethal to the parasite because of the sequential actions of guanine deaminase (GDA) and XPRT that would convert host-derived guanine to XMP, therefore starving the parasites for guanylate nucleotides. We postulated therefore that a viable ∆gmps mutation would require a ∆gda background to divert guanine metabolism through HGPRT or XPRT. We tested this hypothesis by creating a ∆gda null mutant and subsequently inserting the ∆gmps lesion to generate the ∆gda/∆gmps double knockout parasites for which only guanine or guanosine are permissive for growth. These experiments reveal GMPS to be the only purine enzyme that is indispensable to the parasite under all growth conditions and substantiate its utility as a potential antileishmanial drug target.

5H The ATOM complex: gate to the trypanosome mitochondrion

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Mitochondria are bacteria-derived organelles peculiar to all eukaryotic cells. They rely on the import of most of their proteins from the cytosol. The outer mitochondrial membrane (OM) is the first barrier imported proteins have to overcome. In virtually all eukaryotes Tom40 – the main subunit of the translocase of the outer membrane (TOM) – forms the pore through which proteins enter the organelle. The TOM complex contains six more subunits which act as receptors or are involved in the biogenesis and stabilization of the translocase. No orthologues of Tom40 have been found in bacteria and bioinformatics initially failed to identify a Tom40-like protein in trypanosomatids. Taking a biochemical approach we recently discovered ATOM (a rchaic protein t ranslocase of the OM), an essential OM protein strictly required for protein import in trypanosomes. ATOM shares features with bacterial OMP85-like transporters and shows remote similarity to Tom40. The protein forms a channel and resides in a complex of 700kDa. A procedure combining OM proteomics and immunoprecipitations identified six additional subunits of the complex. These proteins are annotated as “hypothetical conserved proteins”, indicating that they do not have apparent orthologues outside the trypanosomatids and supporting the notion of ATOM being the ancestral protein translocation pore. Four of the subunits are essential for parasite growth. Ablation of these proteins causes a variety of protein import phenotypes including the accumulation of precursor proteins, depletion of mitochondrial proteins, reduced import rates in in vitro assays and collapse of the ATOM complex. Our findings suggest that we have identified the core components of the ATOM protein translocase and we are currently aiming to understand their function with regard to import specificity.

5l The essential Leishmania major MAP kinase LmaMPK4 is involved in pH sensing and differentiation of metacyclic promastigotes

***Dacher, Mariko**; Beverley, Stephen M.; Leclercq, Olivier; Morales, Miguel A.; Pescher, Paschale; Spath, Gerald F.*

We studied the role of the L. major MAP kinase LmaMPK4 using a novel knock out system based on the episome pXNG that renders transgenic parasites sensitive to the drug ganciclovir (GCV). LmaMPK4 null mutants established in pXNG-MPK4 transgenic parasites retained pXNG-MPK4 during negative selection despite the toxic effect of the drug, demonstrating an essential role of LmaMPK4 for promastigote viability in culture. Various LmaMPK4 mutants were created and tested for their ability to replace the pXNG-LmaMPK4 WT copy in a “plasmid shuffle” approach that combines negative selection with genetic complementation. First, we confirmed that the TXY motif, required for MAP kinase activation by higher order kinases, is essential for MPK4 function and parasite survive. Likewise, we found that a Leishmania-specific N-terminal sequence of 17 amino acids is required for promastigote viability. Surprisingly, MPK4 null mutants were viable expressing a putative dead mutant form of the kinase (MPK4-K59R). These parasites were normal in promastigote growth and morphology, but showed increased resistance to acidic pH in culture that was associated with a substantial increase in parasites that correspond to bona fide metacyclic parasites in morphology, surface glycolipid composition and density. Nevertheless, these parasites showed virulence attenuation in macrophage infection assays, especially at low multiplicity of infection. Kinase activity assays are currently performed in order to link this phenotype either to increased or attenuated MPK4-K59R activity. The genetic approaches presented here allow new insight into the function of an essential Leishmania protein kinase, which escapes classical knock out analyses due to the lethal null mutant phenotype. Our data reveal that MPK4 phospho-transferase activity may be involved in metacyclic differentiation and virulence.

5J The Leishmania MAP kinase MPK10 is essential for L. donovani axenic amastigote development and regulated by a parasite-specific auto-inhibitory domain.

Cayla, Mathieu (Institut Pasteur)*; Rachidi, Najma (Institut Pasteur); Schmidt-Arras, Dirk (Institut Pasteur); Rosenqvist, Heidi (University of Southern Denmark); Jensen, Ole Nørregaard (University of Southern Denmark); Wiese, Martin (University of Strathclyde); Späth, Gerald (Institut Pasteur)*

We established conditional null mutants and used bacterial and leishmanial expression systems to gain mechanistic insight into function and regulation of the L. donovani MAP kinase MPK10. To guard against a lethal null mutant phenotype, the endogenous MPK10 alleles were deleted in transgenic parasites that expressed the target gene from a negative selectable vector. Negative selection of MPK10-/-[pXNG-MPK10] promastigotes allowed for removal of the episome without overt effect on parasite growth or morphology. These “cured” null mutants however did not survive culture conditions that mimic acidic pH and elevated temperature encountered inside mammalian host cells, and thus failed to convert into axenic amastigotes. Likewise, negative selection of MPK10-/-[pXNG-MPK10] during and after axenic amastigote differentiation resulted in parasite death. Together these data suggest essential functions of MPK10 in environmental-induced development towards amastigotes and intracellular survival. Utilizing transgenic parasites over-expressing a GFP-MPK10 fusion protein, we correlated amastigote-specific functions with stage-regulated kinase activity in axenic amastigotes by in vitro kinase assays. Surprisingly, activity did not correlate with phosphorylation of the regulatory tyrosine residue of the highly conserved kinase activation loop, which showed constitutive phosphorylation. Our data therefore suggest that MPK10 activity is constitutive but potentially controlled by auto-inhibition. Removal of a parasite-specific C-terminal domain indeed caused significant increase in phosphotransferase activity, revealing its role in negative regulation of MPK10 kinase activity. Quantitative phosphoproteomic analysis identified increased phosphorylation of serine 395 in this domain in L. mexicana promastigotes, suggesting regulation of auto-inhibition by phosphorylation. In conclusion, our data unravel essential and stage-specific functions of MPK10, which adopts a “ready-to-go” conformation in promastigotes, likely acting as a molecular signalling switch required for axenic amastigote differentiation.

Talk Session VI: Cell Biology II
(Chair) Luisa Figueiredo

04/24/2013

6A How to evade capture: VSG trafficking and maintaining the African trypanosome surface

Field, Mark F. (University of Cambridge)*; Manna, Paul (University of Cambridge); Boehm, Cordula (University of Cambridge); Gadelha, Catarina (University of Cambridge); Adung'a, Vincent (University of Cambridge); Obado, Samson (The Rockefeller University); Chait, Brian T. (The Rockefeller University); Rout, Michael P. (The Rockefeller University)*

Mono-allelic expression and antigenic variation of the variant surface glycoprotein (VSG) of Trypanosoma brucei spp melds two mechanisms that together constitute an elaborate, but extremely efficient, immune evasion strategy. Extreme levels of VSG expression are also required to enable this mechanism, such that VSG contributes 90% of trypanosome surface proteins; this curious state serves to occlude a high number of surface epitopes from immune recognition. How this most unusual surface is made, maintained and turned over is of significance both to understanding how the VSG immune evasion system operates and also in terms of seeking therapeutic opportunities to disrupt the host-parasite interface. For decades we have known that African trypanosomes have an extreme level of endocytic activity, but the last fifteen years have explained why this may be so, and also described an itinerary for VSG trafficking together with an atlas of the trypanosome endomembrane system. The charting of these routes, their contributions to immune evasion and some important remaining questions for the future will be discussed.

6B Analysis of genetic exchange in experimental crosses of Leishmania major using Next Generation Sequencing Data

Shaik, Jahangheer S. (Washington University in St. Louis)*; Akopyants, Natalia (Washington University in St. Louis); Dobson, Deborah (Washington University in St. Louis); Lawyer, Phillip (National Institutes of Health); Elnaiem, Dia-Eldin (National Institutes of Health); Sacks, David (National Institutes of Health); Beverley, Stephen (Washington University in St. Louis)*

Previously we reported the first experimental cross between genetically marked strains of L.Major (Akopyants et al Science 2009). The progeny generated from matings between the Friedlin,FV1-SAT and LV39c5-HYG showed uniparental inheritance of kinetoplast maxicircle genome and diparental inheritance of parental genomes. The parents differ in their infection profile in mice (FV1/fast, LV39cl5/slow), and the hybrids fell into two groups mirroring either parent, suggestive of 1:1 genetic segregation. In order to comprehensively understand the exchange process at a molecular level, next generation sequencing was used to characterize both the parents and 16 clonal diploid progeny obtained through crosses performed in the natural vector Phleobotomus-papatasi as well as the unnatural vector Lutzomyia-longipalpis. Sequencing was performed using Illumina:Hi-Seq machine with 60x coverage and the reads were mapped to the L.majorFriedlin reference from which aneuploidy and SNP patterns were derived and visualized. As observed in other Leishmania, both parents showed a low degree of aneuploidy (6%). In the hybrids, with a few exceptions (1%), all loci showed both parental alleles. The exceptions were the presence of only one parental chromosome (0.4%) or recombinations leading to loss of heterozygosity (LOH) across large stretches of chromosomes (0.6%). Interestingly a great majority of events were seen in one hybrid emerging from the Lu-longipalpais (75%), suggestive of ‘genomic revolution/catastrophe’. Chromosome number inheritance was complex; often differing from predicted parental karyotype with both ‘gain’ and ‘loss’, in some cases accompanied by LOH. Perhaps due to complexity in chromosome numbers we have not been able to construct genetic-maps. however we have been able to search for SNP associations with the Fast vs Slow virulence profile, with several candidate loci identified.

6C Three dimensional organisation of connections between basal bodies and the kinetoplast.

Towers, Katie (Oxford Brookes University)*; Hughes, Louise; Gull, Keith (Oxford University); Vaughan, Sue (Oxford Brookes University)*

Cilia and flagella are assembled from basal bodies and their duplication is tightly regulated within the cell cycle. Basal bodies exist as a pair with a defined lineage and are physically connected to each other. During S-phase in T. brucei the pro-basal body 'matures', a new flagellum grows alongside the old flagellum and two new probasal bodies assemble next to each mature basal body. There is still a poor molecular and ultrastructural understanding of the connections between the basal bodies and how these are re-organised during basal body duplication and segregation. We employed cellular electron tomography and serial thin section analysis to visualise the three dimensional organisation of basal bodies in T. brucei. We characterised a set of connections which exist between basal bodies at various stages of the basal body maturation cycle. These connections are remodelled during duplication and to enable segregation. We discovered a microtubule-based structure involved in linking the basal body to the kinetoplast. This microtubule linkage is maintained throughout the cell cycle. An additional transient subset of microtubules associates with the kinetoplast as it replicates. This work now paves the way for detailed analyses of defective basal body duplication and segregation in mutant cell lines.

6D A novel component of the flagellum attachment zone (FAZ) and its role in FAZ assembly, cytokinesis, and cell morphogenesis in Trypanosoma brucei

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

The flagellum in Trypanosoma brucei is attached to the cell body via a unique cytoskeletal structure termed the flagellum attachment zone (FAZ) that contains a single proteinaceous filament and a specialized set of four microtubules associated with the smooth endoplasmic reticulum and is required for flagellum attachment and cytokinesis. Despite the crucial roles of the FAZ, the components of the FAZ filament and the mechanism of FAZ assembly remain poorly defined. Here, we report the identification of a novel component of the FAZ, which was designated FAZ2, and its essential roles in FAZ assembly, flagellum attachment, cytokinesis, and cell morphogenesis in the procyclic form of T. brucei . Knockdown of FAZ2 resulted in severe growth inhibition and accumulation of multinucleated cells, suggesting defects in cytokinesis. Moreover, FAZ2 deficiency inhibited the segregation of the duplicated basal bodies and compromises the assembly of the new FAZ, leading to detachment of the new flagellum. Furthermore, at the early stage of RNAi induction, the 2N2K cell appeared to undergo an asymmetrical cytokinesis, producing a normal 1N1K cell with an attached flagellum and a small-sized 1N1K cell with a detached full-length flagellum. Strikingly, the cells with a detached flagellum lose their normal shape and polarity and fail to undergo cytokinesis. These observations suggest that the new FAZ defines the cytokinesis cleavage plane and plays critical roles in maintaining cell morphology. Finally, using the method of proximity-dependent biotinylation, the interacting partners and neighbors of FAZ2 were also investigated.

6E IRON UPTAKE IN TRYPANOSOMA BRUCEI

Taylor, Martin C. (London School of Hygiene and Tropical Medicine)*; McLatchie, Alex (LSHTM); Thomas, James (LSHTM); Ntais, Dionysios (LSHTM); Kelly, John (LSHTM)*

Iron is an essential nutrient in trypanosomes. Bloodstream trypanosomes derive iron from host transferrin via the ESAG6/7 transferrin receptor. Iron is subsequently released from transferrin in the lysosome as FeIII. However the pathway by which FeIII is reduced then transported into the cytosol has not been resolved. The minimal requirement for this process would be a ferric reductase and a cation channel/transporter. We have constructed bloodstream form null mutants of the two transmembrane ferric reductases (FRs) in the T. brucei genome. The FRs are orthologues of cytochrome b561 (TbCytb561) and cytochrome b558 (TbFre1), respectively. Both null mutants show no growth defect in vitro, however the TbCytb561 mutants (and TbCytb561 RNAi lines) are resistant to the iron chelator deferoxamine (DFO), while the TbFre mutants are more susceptible to DFO than the wild type. This apparent paradox is explainable by differential protein localization. We have also identified a candidate for the ion channel, an orthologue of the human endosomal iron-release channel Mucolipin 1. TbMLP (Mucolipin-like protein) is an integral membrane protein which we show is confined to the endolysosomal system. RNAi against the TbMLP1 gene resulted in modest growth retardation, apparent between 3 and 6 days after induction, consistent with previous reports that trypanosomes have an internal iron store. However, when exposed to DFO or salicylhydroxamic acid, the induced cells have a severe growth defect and are hypersensitive. This phenotype is recapitulated in a conditional null mutant. Our current hypothesis is that translysosomal membrane iron transport is mediated by TbCytb561 and TbMLP in concert.

6F Iron regulated modulation in ROS levels: a novel signaling mechanism for Leishmania differentiation.

Mittra, Bidyottam (University of Maryland, College Park)*; Menezes, Juliana (University of Maryland, College Park); Miguel, Danilo (University of Maryland, College Park); Andrews, Norma (University of Maryland, College Park)*

Leishmania alternates between insect and vertebrate hosts, thereby experiencing extreme environmental changes during its life cycle. Some of these shifts in environmental conditions can initiate differentiation into infective forms. For example, elevated temperature and decreased pH, conditions encountered after macrophage invasion, induce axenic differentiation of avirulent promastigotes into virulent amastigotes. Surprisingly, we found that the upregulation of iron transport observed when L. amazonensis are exposed to low iron conditions can also act as a trigger for promastigote-amastigote differentiation, independently of shifts in temperature and pH. Iron depletion from the culture medium induced expression of the ferrous iron transporter LIT1, increase in iron content, growth arrest, and differentiation of wild type promastigotes into infective amastigotes. LIT1 null promastigotes, in contrast, showed reduced intracellular iron and enhanced growth in iron-poor media, followed by massive cell death. Upregulation of iron superoxide dismutase (FeSOD) activity and increased ascorbate peroxidase transcript levels were observed in WT promastigotes grown in iron-poor medium, whereas LIT1 null cells showed lower FeSOD activity and accumulation of superoxide radicals. While both menadione (a superoxide-inducing agent) or H 2 O 2 were sufficient to trigger differentiation of wild type promastigotes into fully infective amastigotes, only H 2 O 2 was able to initiate differentiation of LIT1 null parasites. These findings point to H 2 O 2 , generated from superoxide radicals by Leishmania FeSOD, as the potential signaling molecule for initiating amastigote differentiation. We are currently exploring the roles of individual L. amazonensis FeSODs in this process, and recent findings on the mitochondrial FeSODA will be discussed.

6G Detecting and defining subdomains at the surface of African trypanosomes by proteomics

***Gadelha, Catarina**; Chait, Brian T.; Field, Mark C.; Zhang, Wenzhu*

To perform antigenic variation, the African trypanosome surface coat must be kept free of many essential invariant proteins. These are instead sequestered to the flagellar pocket, a specialised region of the surface membrane that is the sole site of endo/exocytosis. In previous work we and others defined morphological membrane domains and boundaries around the flagellar pocket, identified their association with the internal cytoskeleton, and described how nutrient macromolecules may gain access to the cell interior via a continuous channel linking the extracellular environment to the pocket lumen. However, there remains a paucity of data concerning the molecular composition of the flagellar pocket. This severely hampers understanding of flagellar pocket mechanisms, and also possible exploitation in drug and vaccine development. Using chemical derivatisation of surface membrane, tandem mass spectrometry, quantitative analysis and bioinformatic filters we describe a new surface proteome for bloodstream form Trypanosoma brucei. This set is enriched in GPI-anchored proteins, transmembrane proteins and annotations similar to known surface components. By creating a genetic toolkit for tagging membrane proteins from endogenous loci, we have localised several putative surface molecules of unknown function. Our results validate the surface membrane location of many novel components, and also show that individual proteins can access different combinations of cell body, flagellar and flagellar pocket membranes. We propose that T. brucei exhibits distinct domains on its surface with restricted diffusion between them. This paradigm has important implications for the function of the trypanosome cell surface.

6H BioID for trypanosomes - novel bilobe components identified by proximity to TbMORN1

Morriswood, Brooke; *Havlicek, Katharina; Demmel, Lars; Yavuz, Sevil; Sealey-Cardona, Marco; Vidilaseris, Keni; Kostan, Julius; Djinovic-Carugo, Kristina; Roux, Kyle; Warren, Graham*

The complex and highly-ordered cytoskeleton of Trypanosoma brucei has been shown to play vital roles in its biology but remains difficult to study, in large part owing to the intractability of its constituent proteins. Existing methods of protein identification such as bioinformatic analysis, generation of monoclonal antibody panels, proteomics, affinity purification and yeast two-hybrid screens all have drawbacks. Proximity-dependent biotin identification (BioID) is a recently developed technique that allows forward screens for interaction partners and near neighbours in a native environment, and with no requirement for solubility in non-ionic detergent. As such, it is extremely well suited to the exploration of the cytoskeleton. In this project, BioID was adapted for use in T. brucei. The trypanosome bilobe, a discrete cytoskeletal structure with few known protein components, represented an excellent test subject. Use of the bilobe protein TbMORN1 as a probe resulted in the identification of seven new bilobe constituents and two new flagellum attachment zone proteins. This constitutes the first usage of BioID on a largely-uncharacterized structure, and demonstrates its utility in identifying new components of such a structure. This remarkable success validates BioID as a new tool for the study of unicellular Eukaryotes in particular, and the cytoskeleton in general.

Talk Session VII: Gene Expression
(Chair) George Cross

04/25/2013

7A Characterization of cdc2-related kinase 9 (CRK9), a key enzyme in trypanosome gene expression

Badjatia, Nitika (University of Connecticut Health Center)*; Ambrosio, Daniela (University of Connecticut Health Center); Nguyen, Bao (University of Connecticut Health Center); Günzl, Arthur (University of Connecticut Health Center)*

CRK9 (Tb927.02.4510) is one of eleven cyclin-dependent kinases present in Trypanosoma brucei. Previous results showed that CRK9 silencing was lethal in procyclics causing them to round up like FAT cells that were depleted of tubulin. Since many of these cells had duplicated basal bodies that were incompletely separated, CRK9 may be important in the G2/M phase of the cell cycle. Using the well-controlled pT7-stl RNAi vector, we observed rapid FAT cell formation and cell death upon CRK9 silencing in both procyclic and bloodstream forms. Moreover, we discovered that CRK9 silencing led to a general block of spliced leader (SL) trans splicing and a loss of phosphorylation of the largest RNA polymerase II subunit RPB1. Interestingly, the latter defect did not significantly affect transcription of SL RNA and protein coding genes. Instead, we discovered that CRK9 silencing caused hypomethylation of the SL RNA's cap4 structure that is required for trans splicing. These results demonstrated that CRK9 controls an essential, parasite-specific step in the maturation of each and every mRNA. To characterize this key enzyme, we generated a procyclic cell line that exclusively expressed CRK9 as a fusion with the composite PTP tag and tandem affinity-purified the tagged protein. Although many proteins co-purified with CRK9-PTP, sucrose gradient sedimentation revealed a tripartite complex that was competent in CRK9 autophosphorylation and comprised a novel cyclin, which we termed CYC12, and a conserved hypothetical protein. CYC12 silencing reproduced the CRK9 silencing defects identifying CYC12 as the functional CRK9 partner in gene expression control. Moreover, we recently obtained an RNAi cell line for the third protein; induction of dsRNA synthesis caused the cells to round up suggesting that all three components are functionally important for CRK9 activity in vivo.

7B Characterization of the Small Proteome of Trypanosoma brucei

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Advances in genomics research are providing new avenues to a more holistic understanding of pathogens. An RNA-Seq transcriptome study from our lab identified 1,114 novel transcripts in Trypanosoma brucei of which 993 have at least one potential ORF. The majority fit into the category of short ORFs (sORFs), since the predicted protein is between 25 and 100 amino acids in size. Mining mass spectrometry data sets revealed 42 novel transcripts that encode a sORF matching to at least one unique peptide, suggesting that these proteins are expressed. Thus, the trypanosome proteome appears larger than previously believed. To begin to address the possible function of small proteins in T. brucei, all 42 novel transcripts were down-regulated by RNAi and 7 were determined to be essential in procyclic trypanosomes. Each lethal phenotype was rescued by co-expressing an RNAi-resistant construct, further validating the significance of these small proteins. The 7 essential sORFs are only found in trypanosomatids: five are widespread, while two are specific to African trypanosomes. For example, the essential protein encoded by Tb10.NT87 is 64 amino acids long and localizes to the matrix of the mitochondria, as shown by immuno EM, and a karyopherin-like protein has been identified as a potential interacting partner. On the other hand, Tb11.NT29 encodes 62 amino acids with a predicted trans-membrane domain and is localized on the surface of procyclic- and bloodstream-form trypanosomes. In addition, essential small proteins localize to the nucleolus, cytoplasm, and a perinuclear compartment of the cell, highlighting the diverse biological roles they are likely to play. Experiments are in progress to assess the essentiality in bloodstream-form trypanosomes and to identify interacting partners.

7C VEX1 controls VSG allelic exclusion and antigenic variation in T. brucei

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Antigenic variation in African trypanosomes requires monotelomeric transcription of a single Variant Surface Glycoprotein (VSG) Expression Site (ES) and the concomitant, reversible silencing of a repository of VSGs at silent ESs. The mechanisms underlying activation, silencing and crosstalk among telomeric ESs are not understood. We performed a genome-scale RNA interference library screen for loss of telomeric silencing in bloodstream form T. brucei. From this screen we identified two proteins that play central roles in VSG expression control. The first protein (VEX1 for Vsg EXclusion) is associated with the extranucleolar RNA polymerase I compartment known as the Expression Site Body, while the second decorates a subnuclear compartment that is consistent with a telomeric association. Knockdown revealed loss of ‘basal’ telomeric silencing and loss of VSG silencing in both cases, resulting in cells coated with multiple VSGs. Thus, VEX1 marks the active locus and controls monotelomeric transcription and concomitant VSG silencing underlying antigenic variation and host immune evasion.

7D Chromatin SUMOylation by the SUMO E3 ligase SIZ1 Promotes VSG Expression Site Transcription in T. brucei.

López-Farfán, Diana (IPBLN-CSIC)*; Bart, Jean-Mathieu (IPBLN-CSIC); Navarro, Miguel (IPBLN-CSIC)*

Bloodstream trypanosomes avoid the immune response by periodically exchanging their main surface antigen known as the Variant Surface Glycoprotein (VSG). Transcription of the VSG Expression Site (ES) is mediated by the RNA polymerase I, and localizes to a unique nuclear body named the ESB. In other eukaryotes SUMOylated forms of nuclear proteins work as a seed to assemble and regulate nuclear bodies. To investigate the nuclear localization of SUMOylated proteins in T. brucei, we generated a mouse monoclonal antibody against TbSUMO. SUMOylated proteins were dispersedly located in the nucleoplasm including a discrete and more intense focus. Nuclear co-localization analysis by 3D microscopy showed that the highly SUMOylated area is associated with the ESB nuclear body and the active VSG-ES locus tagged with the GFP. We further investigated the occupancy of SUMOylated proteins within VSG-ES locus by chromatin immunoprecipitation analysis using the anti-TbSUMO antibody. SUMOylated proteins were detected along the active VSG transcription unit, but not in silent VSG-ESs. Chromatin SUMOylation was not significant at either the ribosomal or EP loci, suggesting this is a distinct feature of the VSG-ES regulation. In addition, sequences upstream of the active VSG-ES promoter were highly enriched in SUMOylated proteins. A SUMO E3 ligase (TbSIZ1) was identified by a two-hybrid screen using TbRPB7 as bait, which is required for VSG transcription. Depletion of TbSIZ1 reduced the level of SUMOylation detected in the active VSG-ES chromatin. Furthermore, RNA pol I occupancy and transcriptional activity were decreased upon TbSIZ1 knockdown, suggesting that chromatin SUMOylation is involved in the positive regulation of RNA pol I transcription.

7E Chromatin readers regulate monoallelic expression and switching in T. brucei

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T. brucei employs antigenic variation to evade the host immune system by switching between thousands of variant surface glycoprotein genes (VSG) that are monoallelically expressed from one of ~15 telomeric expression sites. Immune evasion via antigenic variation requires tight control of both monoallelic VSG expression and VSG switching. Since chromatin-interacting factors play a role in both these processes, we tested whether a subclass of proteins responsible for “reading” the histone code have a role in controlling monoallelic expression of VSG proteins. Treatment of trypanosomes with a small molecule that inhibits bromodomain-containing proteins leads to transcriptional changes at silent expression sites, expression of multiple VSGs simultaneously, and a 100-fold increase in VSG switching frequency. Further analysis using RNAi indicates that several of the predicted bromodomain proteins in T. brucei play roles in cell cycle control, maintenance of monoallelic expression, and switching. These studies are recapitulated by follow up experiments with an inducible knockout of the bromodomain protein Bdf2. Specifically, we found that Bdf2 deletion causes cell cycle abnormalities, increased transcription of VSGs at silent expression sites, and an increase in switching frequency. As well, ChIP studies indicate that Bdf2 localizes to expression sites. We are now conducting ChIP-seq studies to localize bromodomain proteins genome-wide. Our studies indicate that bromodomain proteins in general, and Bdf2 in particular, are important for maintaining monoallelic expression and regulating switching frequency, thus providing insight into the molecular mechanism of immune evasion.

7F Histone H1: a linker between chromatin, gene expression and parasite virulence in Trypanosoma brucei

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Histone H1 (H1) is a conserved but poorly characterized component of chromatin in eukaryotes. It binds to the linker DNA between nucleosomes and it promotes condensation of chromatin. Intriguingly, H1 is dispensable for growth of lower eukaryotes, but it is absolutely required for development in mammals. Therefore, it has been proposed that during evolution, H1 might have gained novel functions. Here we characterize histone H1 in Trypanosoma brucei , a eukaryote that has diverged from the main eukaryote branch 300 million years ago. First, we observed that, similarly to other canonical and variant histones in T. brucei , H1 variants are up-regulated in S-phase. When parasites are depleted of H1 chromatin becomes globally more open, but this effect is more pronounced at loci at or upstream the promoters of RNA Polymerase I-transcribed regions. RNAseq showed that H1 controls the expression of a limited set of genes (121 out of 9045 genes), either positively (55 genes) or negatively (66 genes). The majority of loci repressed by H1 are Variant Surface Glycoprotein (VSG) or Expression site-associated genes. Moreover, cells depleted of H1 are more resistant to DNA damage, which is consistent with a role in recombination-mediated VSG switching recently published by the Rudenko lab. Interestingly, although H1 depleted parasites grew almost normally in culture, their virulence in mice was reduced in a dose-dependent manner, indicating that H1 may be more relevant in unicellular eukaryotes than previously anticipated. Overall, our results indicate that T. brucei H1 seems to act by conserved molecular mechanisms, but it is important for virulence in vivo and for the expression of a specific group of genes (VSG gene family).

7G The multiple cap-binding protein paralogs enable Leishmania to survive a multitude of physiological stresses

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Cap-dependent translation is inhibited in response to heat shock in higher eukaryotes, as well as in Leishmania. Thus, alternative pathways must come into play in response to the variety of stress conditions which are encountered throughout the life cycle of the parasite. Earlier attempts identified LIF4E-4 as the canonical eIF4E, which under normal conditions anchors the cap-dependent translation-initiation complex, together with LIF4G-3 and LIF4A1. However, the LIF4E-4 complex disintegrates at elevated temperatures, and the only eIF4E paralog that functions during a thermal stress is LIF4E-1. The LIF4E-1 complex lacks a scaffold protein, but possesses a novel network of interactions that is responsible for recruiting LIF3 and the small ribosomal subunit to the initiation complex. We have obtained crystals of LIF4E-1, in complex with a binding peptide derived from the novel 4E-interacting protein (L4E-IP) that we identified, and is assumed to act as a regulator of LIF4E-1. A less studied nutritional stress is also experienced by Leishmania, which assigned a specific cap-binding protein, LIF4E-3, to overcome its damaging effects. LIF4E-3 hardly binds m7GTP or cap-4, in accordance with the W170M replacement that occurred in its cap-binding pocket. Although LIF4E-3 has an eIF4G binding partner, LIF4G-4, the two proteins do not co-migrate on sucrose gradients. Furthermore, both biochemical analysis and confocal microscopy show that only LIF4E-3 and not LIF4G-4, enters large particles, devoid of DHH1, that increase in size during nutritional stress. We propose that under normal conditions, LIF4G-4 sequesters LeishIF4E-3 in the cytoplasm. During a nutritional stress, LIF4E-3 is modified and released from LIF4G-4 to enter granules which possibly store inactive mRNAs.

7H A DEAD box RNA helicase homolog plays a key role in Leishmania amastigote differentiation and adaptation to intracellular stress

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RNA helicases of the DEAD box family are present in all eukaryotic cells as well as in many bacteria and archaea. These highly conserved proteins are involved in nearly all aspects of RNA metabolism, from transcription and mRNA decay to translation and are therefore considered central players in the regulation of gene expression. We have reported recently that a Leishmania 67 kDa ATP-dependent DEAD-box RNA helicase (HEL67) protects ribosomal RNA (rRNA) from degradation upon stress and induction of apoptosis through a novel mechanism involving antisense rRNA1. Here, we provide functional insights into the role of HEL67 in translation regulation and the intracellular survival of the parasite. We show that HEL67 is associated with polysomes and the translation apparatus and that its ATPase activity is essential for optimal Leishmania translation. Most importantly, we show that genomic depletion of HEL67 interferes with amastigote differentiation and dramatically decreases intracellular survival both in vitro and in vivo. Decreased intracellular survival of the L. infantum HEL67(-/-) null mutant is largely due to its inefficient response to heat stress, an important triggering factor for promastigote to amastigote differentiation. Furthermore, we show that the Leishmania HEL67(-/-) null mutant produces significantly higher levels of reactive oxygen species, which partly explains its high sensitivity to the apoptosis-inducing agent miltefosine. Overall, this study suggests a key role of the HEL67 DEAD box RNA helicase in Leishmania amastigote differentiation and development in the vertebrate host. 1Padmanabhan PK, Samant M, Cloutier S, Simard MJ, Papadopoulou B. Cell Death Differ. 2012 Dec;19(12):1972-82.

7I The role of RNA-binding protein RBP6 in mediating progression through the insect life-cycle stages of Trypanosoma brucei

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T. brucei undergoes an elaborate development within the tsetse fly, culminating with the generation of infective metacyclic forms in the salivary glands. We recently established an in vitro system recapitulating most aspects of the differentiation process in the fly, based on inducible expression of a single gene, RBP6. To gain insight into the molecular mechanisms driving developmental progression to infectivity, we are studying the function of RBP6 by analyses of its structure, RNA-binding preferences and its global effects on the transcriptome. Modeling of the tertiary structure of RBP6 identified a second domain with an RNA Recognition Motif (RRM) fold at the N-terminus of the protein in addition to the previously predicted RRM at the C-terminus. Deletion of the oligoglutamine region linking the two RRMs (which varies in length in African trypanosomes) did not impede the ability of RBP6 to trigger developmental progression leading to the production of metacyclics. In vitro UV-crosslinking studies demonstrated direct contact between RBP6 and an RNA substrate generated by Systematic Evolution of Ligand by EXponential enrichment (SELEX). The SELEX approach yielded the consensus RNA-binding site 5'-CU(U/A)AU(G/A)-3'. The genome-wide effects of RBP6 on mRNA abundance 24 hours after induction of its expression were analyzed using mRNA-Seq. Surprisingly, transcripts that are putative targets of RBP6, based on the presence of a SELEX consensus in their 3'UTRs and co-immunoprecipitation with tagged RBP6, showed little change in abundance upon induction of RBP6 expression and these observations hint to a likely function of RBP6 in regulating translation. Our results underscore the importance of RNA-binding proteins in post-transcriptional regulation of gene expression in trypanosomes and highlight their combinatorial mode of action.

Poster Session: A

04/22/2013

77 Acidocalcisome-mediated autophagic regulation in Trypanosoma brucei

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Trypanosoma brucei has a complex life, proliferating in the midgut of tsetse fly (procyclic form) or the blood stream of mammals (bloodstream form). Each life stage exhibits different morphologies and catabolic or biosynthetic capacities, adapted to the distinct host environments. Autophagy, the bulk degradation pathway to clear macromolecules or whole organelles through double-layered membrane-bound autophagosomes, was reported to be involved in the transformation and starvation response in trypanosomes and was prospected to be a drug target against trypanosomiasis. But the absence of detailed molecular signaling pathway in T. brucei precludes understanding of the mechanisms and targeting of this process for therapy. Our previous work showed that chloroquine (CQ) blocked the autophagosome-lysosome fusion, stopped the clearance of autophagosomes and led to a slight increase of autophagosome number after 2 hours starvation. Intriguingly and distinct to other organisms, Bafilomycin A1 (Baf A1), which has similar function to CQ, completely inhibited autophagy initiation in trypanosomes. We reported here the evidence supporting a role of acidocalcisome in autophagic regulation in procyclic T. brucei . These results suggest a possibly unique acidocalcisomes-mediated mechanism to regulate autophagy via Ca 2+ or H + signaling pathways in trypanosomes.

78 Role of Centrins in Cytokinesis and Organelle Mis-segregation in Trypanosoma brucei

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Centrins are eukaryotic calcium sensor proteins involved in cell cycle. We have earlier shown that depletion of centrin1 in Trypanosoma brucei (T. brucei) displayed arrested organelle segregation resulting in loss of cytokinesis. This study analyzes the role of T. brucei centrin2 (TbCen2) and T. brucei 3 (TbCen3) in the early events of T. brucei cell cycle. Both the immunofluorescence assay and electron microscopy showed that TbCen2 and 3-deficient procyclic cells were enlarged in size with duplicated basal bodies, multinuclei and new flagella that are detached type. In both TbCen2 and TbCen3 depleted cells segregation of basal bodies, kinetoplast and nucleus were disrupted. Further analysis of the cells with defective organelle segregation identified three different sub configurations (Type 1-3). In addition, in majority of the TbCen2 depleted cells and in nearly half of the TbCen3 depleted cells, the kinetoplasts were enlarged and undivided. The abnormal segregations ultimately led to aborted cytokinesis, thereby affecting cell growth. Therefore, both centrin2 and 3 are involved in organelle segregation similar to centrin1. In addition, their role in kinetoplast division has been identified, which may be also linked to overall mis-segregation.

79 Eighteen amino acids at the N terminal tip of Leishmania proline/alanine transporter determine substrate specificity

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Promastigotes of the parasitic protozoa Leishmania proliferate in the proline- and alanine- rich environment of the sand flies mid-gut. The Parasites use these amino acids as alternative carbon sources and as osmolytes. Recently, we have identified a new amino acid transporter, LdAAP24 that translocates proline and alanine across the L. donovani plasma membrane. LdAAP24 contains a long hydrophilic N-terminus of 89 amino acids. Alternative trans-splicing gives rise to two variants of this transporter: the complete ORF of 488 amino acids (LdAAP24a) that translocates both proline and alanine, and an 18 amino acid shorter protein (LdAAP24b) that transports only proline. We hypothesized that the missing amino acids alosterically regulate alanine transport via self-interaction with the internal loops between the trans- membrane domains. Co-expression of the full length soluble N terminus with LdAAP24b in promastigotes that lack LdAAP24 gene rescued alanine transport. Transport rate of alanine in the co-expression cells was similar to that seen in LdAAP24a. Currently, we are mapping the amino acids and investigating the molecular mechanism of this regulation. To our knowledge, this is the first transporter whose amino acid terminal tip regulates substrate specificity.

80 Characterization of the glycerol-3-phosphate acyltransferase TbGAT of Trypanosoma brucei

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Glycerolipids are the main constituents of biological membranes in Trypanosoma brucei, which causes sleeping sickness in humans. Most importantly, they occur as a structural component of the glycosylphosphatidylinositol (GPI) lipid anchor of the most abundant cell surface glycoproteins procyclin in procyclics and variant surface glycoprotein in bloodstream forms that play crucial roles for the development of the parasite in the insect vector and the bloodstream of the mammalian host, respectively. The present work reports the characterization of the unique glycerol-3-phosphate acyltransferase TbGAT that initiates the biosynthesis of ester glycerolipids. Data related to its substrate specificity, subcellular localization, importance in parasite's viability and lipid metabolism will be presented.

81 The role of the flagellum attachment zone in subpellicular microtubule organization

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The cell cycle of the flagellated protozoan Trypanosoma brucei , proceeds with a temporal and spatial coordination of morphogenetic events which are governed by the microtubule cytoskeleton. The subpellicular microtubule array forms the most prominent structural system and remains intact throughout the cell cycle. The flagellum attachment zone (FAZ) plays a crucial role in attaching the flagellum to the cell body, and mediating flagellum regulation of cell morphogenesis. This poorly defined structure contains ?-tubulin, but little is known about its specific function on FAZ. To further the knowledge of microtubule mediated events during T. brucei cell cycle, the dynamics of the microtubule cytoskeleton was visualized by inducible expression of YFP-a-tubulin. In addition to verifying the mode of cytoskeletal replication, this study aims to identify the role of ?-tubulin at the flagellum attachment zone in microtubule organization.

82 The trypanosomal pumilio protein TbPUF2 is involved in the regulation of mRNAs coding for cytoskeletal proteins

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PUF proteins (named after the founding members Pu milio from Drosophila melanogaster and F BF from Caenorhabditis elegans) constitute an important RNA binding protein family in eukaryotes. These proteins bind to the 3'UTRs of their target RNAs and thereby regulate their expression. We are studying the trypanosome pumilio domain protein Tb PUF2. Depletion of TbPUF2 by RNAi inhibits growth of the bloodstream form. The protein is localized in the cytoplasm, which is in accordance with a role in mRNA metabolism. Tb PUF2 is not associated with the actively translating mRNA fraction (polysomes). TbPUF2 RNAi leads to a lower expression of a large number of mRNAs coding for cytoskeletal proteins including the paraflagellar rod proteins 1 and 2. We therefore can expect that the protein might be stabilizing its target RNAs. However, artificial tethering of Tb PUF2 to a reporter mRNA leads to a decrease in the reporter level. This indicates that Tb PUF2 might be involved in the destabilization of its target RNAs. We do not understand this contradiction yet. UV crosslinking followed by immunoprecipitaion of Tb PUF2, showed the RNA binding ability of the protein, however the amount of RNA bound to the protein in vivo was very low. Further analysis by sequencing of the RNAs bound to Tb PUF2 will provide more insight into its target RNAs as well as its possible functions in trypanosomes.

83 Quantitative phosphoproteomic analysis of procyclic and bloodstream form Trypanosoma brucei reveals potential roles for phosphorylation in adaptation

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Trypanosoma brucei has a complex digenetic lifecycle between a mammalian host and an insect vector, and adaption of its proteome between lifecycle stages is essential to its survival and virulence. Here, we report the global quantitative proteomic and phosphoproteomic analysis of bloodstream and procyclic form T. brucei using stable isotope labelling by amino acids in culture (SILAC) of each lifecycle stage. We were able to identify 5,478 proteins and quantify SILAC ratios for 3,763 proteins, with >10% of proteins differentially regulated >5-fold between lifecycle stages, including those involved in the parasite surface coat, and in mitochondrial and glycosomal energy metabolism. We observe usually strong correlation (0.85) between changes in protein levels and the change in mRNA levels reported in previous transcriptomic studies, supporting the hypothesis that the post-transcriptional regulation of mRNA level is a major component in the regulation of gene expression in T. brucei. Phosphopeptide enrichment by SCX and TiO2 lead to the identification of 10,096 phosphorylation sites (>90% new) on 2,551 proteins, and quantified the ratios of 8,275 phosphorylation sites between the two lifecycle stages. Widespread differential protein phosphorylation occurs between bloodstream and procyclic from trypanosomes, with significant intra-protein differential phosphorylation. Gene enrichment analysis of phosphorylated proteins identified GO terms relating to the flagella, protein kinase activity, and the regulation of gene expression. Despite a lack of dedicated tyrosine kinases, phosphotyrosine residues were 3-4 fold over-represented amongst site changing >10-fold between the two lifecycle stages. The majority of the T. brucei kinome was phosphorylated, with evidence that MAPK pathways are functional in both lifecycle stages. The extensive phosphorylation of RNA binding proteins observed may be relevant to the control of mRNA stability.

84 Trypanothione-dependent peroxidases protect African trypanosomes from lysosomal oxidative stress.

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In African trypanosomes, detoxification of hydroperoxides is achieved by 2-Cys-peroxiredoxin and non-selenium glutathione peroxidase-type enzymes (Px) which both obtain their reducing equivalents from the trypanothione/tryparedoxin system. Our previous knockout studies revealed that the two cytosolic Px I and II are essential while the mitochondrial Px III is not. Bloodstream Trypanosoma brucei lacking Px I and II are fully viable in the presence of Trolox. However, removal of the vitamin E analog from the culture medium results in severe lipid peroxidation and cell lysis within a few hours [Diechtierow and Krauth-Siegel, 2011, Free Radic Biol Med, 51, 4]. The exact molecular mechanism of this extremely rapid cell lysis was not clear. In the present work, live cell imaging of the mutant parasites fed with a fluorescent dextran revealed a marked swelling of the lysosome preceding cell lysis. The removal of fetal calf serum from the medium resulted in a prolonged lifespan of the mutant parasites upon Trolox withdrawal suggesting a serum component causing this phenotype. Thus, the effect of exogenous iron and iron-containing macromolecules was studied. Indeed, the cytosolic Px-type peroxidases seem to play an important role in preventing oxidative stress from iron-related endocytosis and protecting the lysosome. Interestingly, the results obtained so far emphasize the lysosome as main cellular compartment producing oxidative stress, not the mitochondrion. Identification of cellular compartments producing oxidative stress is of utmost importance to understand redox-regulated processes and to reveal promising drug targets.

85 Purification of specific mRNPs via the nascent peptide

Inchaustegui Gil, Diana (ZMBH)*; Clayton, Christine (ZMBH)*

The translation efficiencies and stabilities of trypanosome mRNAs are determined by cis-regulatory motifs, which are usually in the untranslated regions (UTRs). The effects of regulatory sequences depend on interactions with RNA-binding proteins (RBPs). Each translating mRNA is expected to associate with several RBPs and to be regulated by their combined effects. Therefore, I aim to purify specific ribosome-associated messenger ribonucleoprotein particles (mRNPs). These particles are composed of the mRNA, ribosomes, translation factors and RNA binding proteins. I purify the translating mRNPs via three streptavidin binding peptides at the N-terminus of the nascent polypeptide, using reporter mRNAs with various 3' UTRs. The purification currently yields 16% of the reporter mRNA present in the polysomes, with eight-fold enrichment compared to tubulin mRNA, an abundant mRNA which binds unspecifically to the purification matrix. Quantitative mass spectrometry of the reporter mRNA containing the EP 3'UTR from procyclic trypanosomes revealed enrichment of proteins of the degradation machinery such as XRNA and NOT1, other proteins associated with mRNPs, like MKT1, and also a protein that has not been identified before in other purifications, ZC3H22. In order to validate this technique I am comparing the proteins bound to polysomal mRNAs from reporters with different 3' UTRs. The advantage of this method is that it allows the purification of all proteins bound to a particular UTR and proteins that can regulate the fate of individual mRNAs. Also this technique can be applied to any eukaryotic system.

86 Sterol biosynthesis in Leishmania

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In humans and animals, cholesterol is an essential membrane component and the precursor to steroid hormones. Unlike mammalian cells, fungi and trypanosomatids primarily synthesize ergostane - based sterols such as ergosterol. Functions of sterol metabolism in unicellular organisms are not well understood, which limits further development of drugs targeting this pathway. Here we investigated the role of sterol C14a-demethylase (C14DM) in Leishmania parasites. C14DM is a cytochrome P450-dependent enzyme and the primary target of azole drugs . In Leishmania , genetic or chemical inactivation of C14DM led to a complete loss of ergostane - based sterols and accumulation of 14-methylated sterols. Null mutants of C14DM (c14dm^{-}) were viable and replicative in culture but showed severely attenuated virulence. Expression of GPI-anchored membrane molecules such as LPG and GP63 were also significantly altered in c14dm^{-} mutants . In addition, these mutants exhibited cytokinesis defect and hypersensitivity to heat stress. Further studies indicated that heme-binding was critical for the themotolerance and catalytic activity of C14DM. Together, these findings suggest that the accumulation of 14-methylated sterol intermediates and/or the depletion of ergostane - based sterols are hazardous to Leishmania parasites. Further characterizations of the sterol biosynthesis enzymes will provide novel insight into the role of this pathway in Leishmania infection, and the new knowledge may help improve the efficacy of current antileishmanial drugs.

87 Translational activator complex in mitochondria of Trypanosoma brucei

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The 45S SSU* RNP complex, found earlier in Leishmania tarentolae, represented a heterodimer of the 25-30S small subunit (SSU) of mitochondrial ribosomes with a comparable size protein moiety. The complex contained 9S SSU rRNA, small subunit ribosomal proteins, several pentatricopeptide repeat (PPR) proteins, and proteins not typically found in ribosomes, including rhodanese-domain protein (Rhod) and a large (200 kDa) coiled-coil protein. To investigate the function of this complex, several of its components (Rhod, 200 kDa, PPR29, S17) were ablated by RNAi in T. brucei. A severe growth retardation phenotype, inhibition of mitochondrial protein synthesis of COI and Cyb and reduction in amount of the 45S SSU* complexes were observed as early as 48 h post induction. Relative amounts of translation-competent (long polyAU-tailed) mRNAs of Cyb and COI were reduced and formation of the 80S translation complexes identified by association of the long-tailed mRNAs with the 50S mitoribosomes was disrupted. On the other hand, the long-tailed edited RPS12 mRNA was not substantially affected, and there was no noticeable effect on the RPS12 translation complexes. We propose that the 45S SSU* complex represents a translational activator required for translation of some (e.g. COI and Cyb) but not all (e.g. RPS12) mRNAs.

88 The flagellar pocket collar protein 4 (FPC4): A BILBO1-microtubule linker?

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T. brucei has an essential structure called the Flagellar Pocket (FP), which is the unique site of endo- and exocytosis. The FP is essential for parasite survival and pathogenicity and consists of several distinct structural sub-domains, the least explored being the Flagellar Pocket Collar (FPC). The FPC is located at the neck of the FP. Selective confinement of proteins within the flagellum membrane, FP or surface membranes imply that they are filtered through the FP and/or the FPC then retained. However, the mechanisms involved are unknown. The FPC contains numerous uncharacterised proteins, but one protein, identified in 2008, called BILBO1 is essential for parasite survival, (Bonhivers M. et al, PLoS Biol. 2008 May 6;6(5):e105). A two-hybrid screen was done using BILBO1 as bait versus a T. brucei genomic DNA library. This revealed a number of potential BILBO1 partners. Here we have identified a BILBO1 binding protein, we call FPC4, which localizes to the FPC and forms a ring-like structure by antibody labelling and GFP tagging. Using Y2H technology we have analysed which FPC4 domains are involved in BILBO1 interaction. RNAi of FPC4 induced a weak FP segregation phenotype and abnormal FPC formation in procyclic cells, but dominant negative phenotypes were obtained by long overexpression of GFP tagged FPC4. These cells showed dramatic malformation of the FPC, which appeared as linear polymers within the cytoplasm, rather than rings, that circumvent the flagellum, and resulted in malformation of the FP and cell death. Finally, when expressed in mammalian cells, FPC4 binds to microtubules, suggesting that in vivo, within the parasite, FPC4 may link the FPC to the sub-pellicular microtubule cytoskeleton.

89 Insights into the nuclear mRNA export machinery of Trypanosoma brucei

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We are investigating the process of nuclear mRNA export as a potential additional way of post-transcriptional gene regulation in Trypanosoma brucei. The active translocation of mature mRNA from the nucleus to the cytoplasm is well understood in higher eukaryotes ranging from yeast to human. These model organisms belong to the eukaryotic supergroup of Opisthokonta. Their export factors such as the TREX complex and the export receptor Mex67-Mtr2 are well described and appear to be highly conserved. Kinetoplastids belong to the supergroup of Excavata and the high divergence between Opisthokonta and Excavata makes it difficult to identify orthologous export factors based on sequence similarity. One of the few conserved factors is the ortholog of yeast Mex67. Here, we report the functional characterization of TbMex67. We show that downregulation of TbMex67 by RNAi affects cell growth and leads to a nuclear retention of bulk mRNA. In contrast to all known orthologs, Mex67 of kinetoplastids contains a N-terminal zinc finger motif - a parasite-specific feature that is indispensable for the function of TbMex67. Overexpression of mutated versions of TbMex67 causes a dominant negative effect indicating that essential interacting proteins are sequestered. We used tandem affinity purification of PTP-tagged TbMex67 to identify such proteins and isolated two candidates that are essential for mRNA export as well. TbMtr2 interacts with TbMex67 to form the stable export receptor Mex67-Mtr2. TbIMP in contrast, belongs to the family of importins and is required for shuttling of TbMex67. Our data show that the heterodimeric export receptor is conserved throughout the eukaryotic kingdom. However, the zinc finger motif is unique to kinetoplastids and may play a parasite specific role.

90 Genome-wide mapping of histone H3K4 trimethylation and gene expression regulation in Leishmania

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Drug resistance in Leishmania induces major structural rearrangements of the genome such as gene copy number variation and changes in ploidy. Previous genomic and transcriptomic analyses highlighted a strong correlation between RNA abundance and gene copy number in L. infantum antimony- and methotrexate-resistant parasites. However, some discrepancies were observed for genes distributed on the same chromosome and these discrepancies might be due to gene expression regulation at the chromosomal level. The mechanism of transcription initiation in Leishmania is poorly understood but recent studies pinpointed the importance of histone and DNA modifications in the regulation of gene expression. Using LC-MS/MS analysis, we identified histone modifications in Leishmania and found several methylated and acetylated residues in the core domain of histones as well as in their N-termini and C-termini. We also investigated the genome-wide regulation of transcription in response to the parasite's differentiation and drug resistance development using chromatin immunoprecipitation followed by deep-sequencing of enriched DNA fragments (ChIP-Seq), which maps the precise location of the trimethylation state of lysine 4 of histone H3 (3meH3K4). This epigenetic mark is well-defined in mammals and is associated with active transcription, while in Trypanosoma brucei this modification is localized at the start site of divergent polycistronic transcription units. ChIP-Seq experiments were carried out with the two parasite life stages and with resistant parasites. Our preliminary analysis indicates that 3meH3K4 is enriched in the divergent polycistronic transcription units in the Leishmania genome and that differences exist between the strains studied. A complete analysis of our epigenetic study will be presented.

91 Inhibition of nucleotide sugar transport in Trypanosoma brucei alters surface glycosylation

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Nucleotide sugar transporters (NSTs) are indispensible for the biosynthesis of glycoproteins by providing the nucleotide sugars needed for glycosylation in the lumen of the Golgi apparatus. Mutations in NST genes cause human and cattle diseases and impaired cell walls of yeast and fungi. Information regarding their function in the protozoan parasite, Trypanosoma brucei, a causative agent of African trypanosomiasis, is unknown. Here, we characterized the substrate specificities of four NSTs, TbNST1-4, which are expressed in both the insect procyclic form (PCF) and the mammalian bloodstream form (BSF) stages. TbNST1/2 transport UDP-Gal/UDP-GlcNAc, TbNST3 transports GDP-Man, and TbNST4 transports UDP-GlcNAc, UDP-GalNAc and GDP-Man. TbNST4 is the first NST shown to transport both pyrimidine and purine nucleotide sugars and is demonstrated here to be localized at the Golgi apparatus. RNAi-mediated silencing of TbNST4 in PCF caused underglycosylated surface glycoprotein EP-procyclin. Similarly, defective glycosylation of the variant surface glycoprotein (VSG221) as well as the lysosomal membrane protein, p67 was observed in ΔTbNST4 BSF T. brucei. Relative infectivity analysis showed that defects in glycosylation of the surface coat resulting from TbNST4 deletion were insufficient to impact the ability of this parasite to infect mice. Notably, the fact that inactivation of a single NST gene results in measurable defects in surface glycoproteins in different life cycle stages of the parasite, highlights the essential role of NST(s) in glycosylation of T. brucei. Thus, results presented in this study provide a framework for conducting functional analyses of other NSTs identified in T. brucei.

92 Clu(eless) proteins in *Trypanosoma brucei* – Their enigmatic function

***Benz, Corinna (University of South Bohemia)**; Lukeš, Julius (University of South Bohemia)*

Clu (Clu stered mitochondria) proteins are highly conserved and found in various organisms ranging from yeast to humans. Their function was first analysed in the ameboid protozoan Dictyostelium discoideum , where CluA depletion results in mitochondrial clustering by affecting organellar fusion and fission dynamics. In Drosophila melanogaster , the Clu homologue clueless was shown to associate with mitochondria and affect both their function and localisation. Additionally, genetic interaction with Drosophila parkin , a homologue of the human protein involved in autosomal recessive juvenile Parkinson's disease makes clueless a highly interesting protein to study. The Trypanosoma brucei genome encodes two proteins with a Clu domain, which we have termed Clu1 and Clu2. Depletion of Clu1 in procyclic form cells by RNAi causes a rapid growth arrest within two days of induction. Somewhat surprisingly, the most prominent phenotype we observe is detachment of the parasite flagellum. While this flagellar detachment is accompanied by a loss of directional motility the detached flagella continue beating, even in multinucleate cell aggregates that have lost their normal morphology. Functional analysis of Clu2 in procyclics, detailed structural analysis of Clu1 mutant parasites, localisation and interaction studies of Clu1 as well as functional analyses of both proteins in bloodstream form cells are ongoing and results will be presented.

93 MRE11 involvement in DNA repair and drug resistance in Leishmania

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The protozoan parasite Leishmania modulates the copy number of its genes (and thus their expression levels) through the formation of extrachromosomal DNA amplicons as a mechanism of drug resistance. During gene amplification, rearrangements between direct or inverted DNA repeated sequences (RS) lead to the formation of circular and/or linear amplicons containing genes implicated in resistance. We hypothesize that the linear amplification involves the formation of a DNA break nearby the RS, which is further repaired by homologous recombination after annealing between the RS. We investigated the role of the MRE11 gene, which codes for a putative DNA-binding protein involved in DNA break repair. To investigate the role of MRE11 in the formation of linear amplicons, and hence in drug resistance, we generated Leishmania infantum MRE11 null mutants. We observed a growth delay and a higher sensitivity to DNA damaging agents (methylmethane sulfonate and hydroxyurea) in the MRE11-/- strain, compared to the wild-type cells. These phenotypes were partially reverted when the MRE11 null mutants were complemented with an episomal MRE11 construct, thus suggesting the involvement of MRE11 in DNA repair in Leishmania infantum. We further compared the generation of PTR1 linear amplicons in wild-type, MRE11-/- and complemented strains resistant to methotrexate, a model antifolate drug known to induce the amplification of the PTR1 gene. Consistent with our hypothesis, the MRE11-/- parasites exhibited much lower PTR1 linear amplification than the wild-type strain, suggesting a significant involvement of MRE11 in the formation of linear amplicons. The characterization of MRE11 contributes to increase our understanding of the mechanisms underlying the formation of linear amplicons as important players in the development of resistance in Leishmania.

94 Para Tryp Research Organization

Lyda, Todd A. (Para Tryp Research Organization)

Para Tryp Research Organization is a newly formed non-profit organization focused on fueling bench to bedside biomedical research for the tri-Tryp diseases (T. brucei , T. cruzi , and Leishmania). Awards in the areas of Development, Discovery, Supplement and Travel will soon be available for researchers involved in tri-Tryp disease research. Please visit our website (paratryp.org) for more information about our growing organization. Feel free to stop by, leave your contact information and add your lab to our growing list of paratrypers.

95 Prospects and challenges for repositioning drugs and drug candidates against kinetoplastid diseases

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Drug repurposing for new indications is increasingly prominent as a strategy in industry and government drug discovery and development programs. Successes are well-documented, such as the rescue of eflornithine for use in T. brucei gambiense infection, and the mobilization of medicinal chemistry strategies from hypertension to HIV infection. Since the elucidation of kinetoplastid genomes nearly 10 years ago it has been possible to comprehensively identify parasite homologs of human drug targets. This makes it possible to predict opportunities for repurposing drugs and chemistry for use against these pathogens. However, a review of the literature indicates that the number of well-characterized, high potency drug-like compounds against kinetoplastids remains relatively small. In the interest of assessing the challenges and prospects for success in this field we have revisited the prediction of potential parasite drug targets, and the results reported to date from programs seeking drugs against these targets. From this assessment we propose several priorities for future research, and approaches to improve hypotheses and hypothesis-testing relevant to repurposing. We also recommend a wider practice of data sharing and posting of lessons-learned, especially from studies that do not result in promising molecules - results that we believe are under-reported in the literature.

96 A specific life-cycle stage of Trypanosoma brucei exhibits social motility

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When Trypanosoma brucei procyclic forms are pipetted onto the surface of an agarose plate, they exhibit social motility (SoMo) in a manner reminiscent of bacteria such as Myxococcus xanthus. After a few days incubation, parasite communities form “fingers” that radiate out from the site of inoculation on the plate and are capable of sensing and avoiding each other. We hypothesized that the parasites’ behaviour on plates might correspond to an event in their life cycle. At least two migrations occur in the tsetse fly: first, the movement by procyclic forms from the midgut lumen into the ectoperitrophic space, and second, the migration from the ectoperitrophic space across the cardia to the salivary glands. The trypanosomes found in the midguts of freshly infected flies (early procyclic forms) express two major proteins on their surface, the GPI-anchored procyclins EP and GPEET. When the parasite reaches the ectoperitrophic space, GPEET is down-regulated and the cells express only EP (late procyclic forms). At present GPEET is the only marker to distinguish between early and late procyclic forms. We were able to show that SoMo is a property of early procyclic forms and that trypanosomes become SoMo-negative when they differentiate into late procyclic forms. GPEET is not essential for SoMo, however, because GPEET null mutants are still SoMo-positive. This indicates that other differentially regulated proteins are likely to be responsible for the SoMo phenotype. Several mutants that show defects in establishing salivary gland infections exhibit normal SoMo. These results suggest that SoMo corresponds to an early event in midgut infection, most probably the migration from the lumen to the ectoperitrophic space.

97 Identification of the mRNA-fate modulators through a high-throughput tethering assay

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The regulation of mRNA decay and translation is central to post-transcriptional regulation of gene expression in trypanosomatids. A complete understanding of these dynamic processes will be possible only if we identify all the trans-acting factors involved. Genome-wide screens have proven powerful in associating gene products with certain phenotypes or signal transduction pathways, and thus are valuable tools to ascribe gene function. Methods for high-efficiency transfection have made it possible to perform systematic genome-wide functional screens. We intend to discover genes that regulate mRNA and protein abundance. Here, we report on the application of an overexpression screen to identify the full set of mRNA-fate regulators. Specifically, we adapted a high-throughput tethering assay which relies on stabilization of a reporter. We have used the screen to uncover genes that, when overexpressed, increase the expression of the reporter gene, either by mRNA stabilization or enhancement of translation efficiency. Our findings reiterate the role that well-characterized proteins play in the regulation of the mRNA fate, and suggest new roles for previously uncharacterized and ‘‘hypothetical’’ genes. In addition, our screen provides a fine structure mapping of the hit candidates’ functional domains. We are currently verifying the functions of several novel candidate regulators. In addition, we are extending the screen to look for proteins that destabilize mRNAs or inhibit translation. We hope that our work will help us to decipher the codes that control gene expression, while also providing a resource to further understand the function of the whole African trypanosome genome.

98 Initiation of nuclear DNA replication in Trypanosoma brucei: a single factor or a complex?

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Until recently, the process of initiation of nuclear DNA replication in Trypanosoma brucei had been poorly studied. Early work suggested that initiation of DNA replication in this parasite might be carried by a single initiator factor, TbORC1/CDC6, and thus may more closely resemble the machinery found in archaea than the one found in most eukaryotes, where a six-subunit Origin Recognition Complex (ORC) is used. Recently, two studies have identified four factors that interact with TbOrc1/Cdc6 and bear limited homology with ORC proteins. Of these, Orc1b was identified by homology with TbORC1/CDC6, while OL1 appears to be a divergent orthologue of the Orc4 subunit. In contrast, OL2 and OL3 cannot be assigned orthology with any eukaryotic ORC subunits and may be kinetoplastid-specific factors. We aim to elucidate how DNA replication initiates in T. brucei. One aspect of this is to determine the topology of interactions amongst these putative ORC factors, including whether they act as a diverged ORC. Through immunofluorescence and EdU detection techniques we have shown that like TbOrc1/CDC6, OL1 and OL3 localise to the nucleus throughout the cell cycle and are detectable in similar puncta. Conversely, Orc1b shows a dynamic nuclear localisation, being only detected in S-phase cells, suggesting that it may be a regulatory factor rather than a static member of T. brucei's ORC-like complex. Induced RNAi silencing of each factor, combined with EdU detection, implicates all four factors in DNA replication, with Orc1b's silencing resulting in the most striking perturbation of replication and growth. S-phase restriction of a putative ORC-like factor may indicate pronounced divergence in the machinery and regulation of DNA replication in T. brucei.

99 An approach to determine the Transcriptome of T. b. rhodesiense from Sleeping Sickness Patients in Uganda

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To date, all expression-profiling studies of African trypanosomes have been performed on cultured parasites or high-density mouse infections. The extent to which these parasites are representative of a real human infection is still not known. Therefore this study is aimed at analyzing the transcriptomes of clinical isolates of T. b. rhodesiense from patient peripheral blood and cerebro-spinal fluid (CSF), using high throughput RNA sequencing technology. One aspect of our work is to analyze the effects of various methods to isolate the trypanosomes from blood on steady state levels of mRNA. Using an infected rodent model, we are testing the effects of red blood cell lysis, DEAE chromatography and differential centrifugation (buffy coat isolation) on the transcriptomes of trypanosomes, in comparison with cultured cells. Sequencing of these samples is in progress and we hope to be able to present the results. Given the low parasitaemias during infection of humans, and therefore the very small amounts of RNA relative to lymphocyte RNA in human samples, there is a need to specifically amplify the trypanosome RNA above the human cellular RNA background. Using the spliced leader sequence that is attached to the 5’ end of all trypanosome mRNAs (following trans-splicing), we have developed a protocol to specifically amplify nanogram concentrations of trypanosome RNA against a background of microgram amounts of human RNA. We are now starting to apply this method to human patient samples. Analysis of the differential gene expression of T. b. rhodesiense in the bloodstream and CSF will provide an insight into the human-trypanosome interaction. Differences between blood and CSF trypanosome transcriptomes may also indicate differences in metabolism which might influence the effectiveness of drugs that target the late stages of Sleeping Sickness.

100 Inositol metabolism in *Trypanosoma brucei*

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Bloodstream form Trypanosoma brucei utilise inositol to make a variety of lipids including phosphatidylinositol, glycosylphosphatidylinositol (GPI) anchors and signaling molecules (PIPs). The kinetoplastid utilises two sources of inositol – uptake from the blood, and de novo synthesised. The de novo synthesis of myo-inositol is essential for the survival of bloodstream form T. brucei because it is preferentially used for the formation of phosphatidylinositol in the endoplasmic reticulum for GPI biosynthesis (Martin and Smith, 2006). This makes the de novo synthesis of myo-inositol an attractive drug target. We will present our exciting new findings on the enzymes involved as well as results from our various screening approaches for compound binding (Major and Smith, 2011) and inhibition of de novo inositol synthesis. Major, L. L. and Smith, T. K. Molecular Biology International Volume 2011, Article ID 389364 K. L. Martin and T. K. Smith, Molecular Microbiology, vol. 61, no. 1, pp. 89–105, 2006. This work is funded by the Wellcome Trust.

101 Mechanistic insights of SIDER2 retroposon-mediated mRNA decay in Leishmania

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We have shown previously that truncated versions of formerly active retroposons, SIDER2 (Short Interspersed DEgenerate Retroposons), located mainly in 3'UTRs of Leishmania transcripts, promote mRNA decay by a novel mechanism involving endonucleolytic cleavage without prior deadenylation. Endonucleolytic cleavage occurs at the beginning of the second conserved 79-nt SIDER2 signature sequence. Mutagenesis studies to alter sequence or structure within the putative cleavage region were carried out and confirmed the importance of secondary structure for endonucleolytic cleavage and mRNA decay. Integration of a reporter gene into genomic loci regulated or not through the SIDER2 pathway revealed that only transcripts harboring an active SIDER2 element were subjected to a rapid decay in a species and a stage-specific manner. We further showed that introducing a hairpin structure upstream of a reporter gene regulated by SIDER2 blocked translation and increased reporter transcript accumulation, suggesting that SIDER2-mediated decay requires ongoing translation. We are currently searching for protein factors involved in SIDER2-mediated mRNA decay using a tethering approach based on the co-transfection of the bacteriophage MS2 coat protein with its cognate RNA hairpin located upstream of a SIDER2-harboring 3'UTR as part of a reporter gene construct. Also, in situ hybridization studies are under way to assess if SIDER2-mediated mRNA decay takes place in a specific cytoplasmic location as opposed to non-SIDER2 stable transcripts. Collectively, these studies provide new insights into the SIDER2-mediated decay mechanism of unstable transcripts in Leishmania.

102 Life without a diamine transporter: a *T. cruzi* perspective

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Polyamines (putrescine, cadaverine, spermidine, spermine) are essential nutrients in all living organisms including Trypanosoma cruzi . This parasite, however, lacks the first enzymatic step of the canonical polyamine pathway and cannot synthesize putrescine de novo; T. cruzi polyamine transport, therefore, plays an indispensable nutritional function by supplying host polyamines to fulfill the parasite polyamine pool, and enabling the survival and proliferation of this pathogen. We have previously identified in T. cruzi , a high affinity putrescine-cadaverine transporter, TcPOT1, which accounts for the capacity of the parasite to transport these diamines. To assess the contribution of this transporter to the overall parasite diamine transport capability, as well as parasite growth and infectivity, a ?tcpot1 null mutant cell line was generated by targeted gene replacement. As anticipated, the ?tcpot1 mutant lacked high-affinity putrescine-cadaverine transport capability but was able to transport diamines by a non-saturable mechanism. Spermidine and arginine transport were not compromised in the ?tcpot1 line confirming the TcPOT1 transporter specificity towards diamines. Although the ability to infect Vero cells was comparable between wild type and the ?tcpot1 parasites, the mutant exhibited a reduced capacity to proliferate; despite the presence of residual spermidine and spermine transporter, the loss of high-affinity diamine import impacted parasite proliferation. These results provide insight into intracellular polyamine availability and suggest a restricted host polyamine environment.

103 Mouse bloodstream infection and pathogenesis is unaffected by disruption of Trypanosome propulsive motility.

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The flagellum of Trypanosoma brucei is an essential and multifunctional organelle that drives parasite motility and is receiving increasing attention as a potential drug target. In the mammalian host, flagellar motility is suspected to contribute to infection and disease pathogenesis. However, it has not been possible to directly test this hypothesis owing to the lack of motility mutants that are viable in the bloodstream-form life cycle stage that infects the mammalian host. We recently identified a bloodstream-form mutant in 427-derived, BSSM T. brucei that has severely defective propulsive motility but is viable in culture. We have now used this mutant to assess the influence of trypanosome motility on infection in mice. By using conventional methods and by developing a live-cell imaging approach to examine parasites in blood and tissues from infected mice we surprisingly find that disrupting propulsive parasite motility has no discernible effect on T. brucei bloodstream infection. Infection time-course, maximum parasitemia, number of waves of parasitemia, clinical features and disease outcome are indistinguishable between motility mutant and control parasites. Parasites in brain tissue were largely confined within blood vessels. Our studies represent important contribution to understanding pathogenic mechanisms and provide new systems for directly examining host-parasite interactions. Funding:NIH-NIAID R01 AI052348 (KLH); Burroughs Wellcome Fund (KLH), Shapiro Fellowship (NKK), UCLA Dissertation Year Fellowship (NKK, KSR), Laventis Fellowship (GL).

104 Expression GPIomics of the Mammal-Dwelling Stages of Trypanosoma cruzi

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Glycosylphosphatidylinositol (GPI)-anchoring is a protein post-translational modification ubiquitously found in eukaryotes. There is a growing body of evidence showing that protein-free GPIs (or glycoinositolphospholipids, GIPLs) and GPI-anchored proteins (GPI-APs) are involved in host-protozoan interaction processes, such as host-cell adhesion and invasion, and pathogenesis. Here, we used a highly sensitive and objective approach that employs liquid chromatography-tandem mass spectrometry (LC-MSn) for the analysis of the GPIome (GPIomics) of the mammal-dwelling trypomastigote and amastigote stages of Trypanosoma cruzi, the causative agent of Chagas disease. This approach allows for the structural characterization of both the lipid and the glycan moieties of GPIs. We have so far identified over 150 GIPL species from these two parasite forms, most of which had not been described in the literature. In contrast to epimastigote-derived GIPLs (eGIPLs), trypomastigote-derived GIPLs (tGIPLs) tend to have longer glycan moieties, many of which contain pentose (xylose) residues. On the other hand, intracellular amastigote-derived GIPLs (aGIPLs) contain sugar moieties that tend to be somewhat less diverse, although on average they are also larger than the ones found in eGIPLs. The lipid moieties of tGIPLs are composed mainly of ceramides, typically with longer fatty acid chains than those of eGIPLs. Conversely, GIPLs tend to have mostly 1-O-alkyl-2-O-acyl-glycerolipid moieties. These traits are probably related to the specific needs of each life-stage. We are currently conducting the proteomic and GPIomic analyses of the GPI-APs derived from these two mammal-dwelling life-cycle stages, which will help us further understand possible structure-function correlations of GPIs and their role in the chronic infection.

105 Involvement of a mitochondrial carrier in drug resistance in T. brucei

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Choline is an essential nutrient in eukaryotic cells, where it can be used as precursor for the synthesis of choline-containing phospholipids. Recent experiments in our lab showed that uptake of choline in T. brucei procyclic and bloodstream forms is carrier-mediated and can be blocked by HC-3, a known inhibitor of choline transport in mammalian cells. To study the importance of choline uptake on viability of T. brucei parasites, we investigated the effects of several choline analogs, which have previously been shown to inhibit choline uptake in Plasmodium and Leishmania and are toxic for these parasites, including T. brucei bloodstream forms. We now found that all compounds tested also block choline uptake in T. brucei bloodstream and procyclic forms. However, the mechanism of drug action is unlikely to occur via inhibition of choline transport, but rather to involve disruption of mitochondrial function. By using an RNAi library to screen for proteins involved in drug import or action, we identified a candidate mitochondrial carrier belonging to the MCP family. Down-regulation of the carrier by RNAi had no effect on parasite growth in culture; however, it prevented drug-induced loss of mitochondrial membrane potential and conferred 8-fold resistance of T. brucei bloodstream forms to choline analogs. Conversely, over-expression of the carrier increased parasite susceptibility more than 13-fold. Immunofluorescence microscopy confirmed the mitochondrial localization of the carrier. Together, our data demonstrate that the identified member of the MCP family is a mitochondrial carrier involved in drug uptake in T. brucei.

106 Mismatch repair in Trypanosoma brucei roles in protection against oxidative stress

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Cells are continuously exposed to intracellular and extracellular mutagens, which can damage several molecules, including DNA, and mutagen defence and repair mechanisms have evolved. Mismatch repair (MMR) corrects mismatched bases during replication, as well as mismatches caused by some base modifications. Thus, MMR is important to avoid some mutagenesis and maintain genome fidelity. In T. brucei , MMR core functions are carried out by bacterial MutS and MutL homologues, working as heterodimers: MSH2a (MSH2-MSH3) and MSH2ß (MSH2-MSH6), and MLH1-PMS1, respectively. To date, only MSH2 and MLH1 function have been examined and only in bloodstream form (BSF) T. brucei cells. We have now generated null mutants of MSH2 and MLH1 in procyclic form (PCF) cells, and MSH3 and MSH6 in BSF. Characterization of tolerance to DNA methylation damage, using MNNG, and evaluating microsatellite stability (MSI) shows that each gene acts in MMR in both the life cycle stages, with the exception of MSH3, which shows no phenotypes. Mutants were also analyzed for their action towards oxidative stress in both the life stages and, remarkably, we find life cycle stage differences, with MSH2 mutants displaying H 2 O 2 sensitivity and resistance in the BSF and PCF, respectively. We suggest that resistance to H 2 O 2 in the PCF is due to cell adaptation during the loss of MSH2 . To test this adaptation to oxidative stress we have re-expressed MSH2 which shows that while MNNG tolerance and MSI are comparable to heterozygous mutants, the response towards oxidative damage is not reverted. MSH6 mutants display tolerance to alkylation damage, but neither MSH3 nor MSH6 seems to have a role in response towards oxidative damage.

107 Characterization, expression and proteolytic activity of Trypanosoma rangeli Major Surface Proteases (MSPs)

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Major Surface Proteases (MSPs or gp63) play a major role in trypanosomatids nutrition, survival, and infection. In Trypanosoma rangeli, a non-pathogenic trypanosome which is able to infect humans, a gene coding for an MSP (TrMSP) has been cloned and sequenced by our group. Thus, the aim of this study is to characterize and determine the expression of transcripts and the proteolytic activity of TrMSPs. TrMSP gene codes for a predicted 588 amino acid protein and has all elements required for its processing and expression in the parasite surface. TrMSP protein has 35% and 58% identity with L. major MSP (LmMSP) and T. cruzi MSP (TcMSP), respectively. TrMSP possesses the HEXXH motif which corresponds to a region previously associated with the MSPs catalytic site. Homology modeling of TrMSP demonstrated a high degree of structural conservation between LmMSP and TcMSP. However, the determination of surface charges of the structures revealed that TrMSP is predominantly positive; in contrast with LmMSP and TcMSP which are predominantly negative. Expression of TrMSPs transcripts was confirmed by the detection of the expected product in RT-PCR assays. Protease activity was confirmed by zymography assays, where it was possible to observe two bands with gelatinase activity in T. rangeli. However, this activity is much lower than in extracts of L. major CC1. Protease activity in T. rangeli and L. major protein extracts was inhibited by phenanthroline. Differences in the protease activity can be explained, at least in part, by the distinct surface charges of the proteins. This study opens new perspectives on MSP function in a non-pathogenic trypanosome to humans, but actively circulating in South America. Supported by FAPEMIG.

108 17-AAG INDUCES INCIDENTAL CELL DEATH OF Leishmania amazonensis WITH AUTOPHAGIC FEATURES

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Leishmaniasis is a neglected endemic disease with a broad spectrum of clinical manifestations. Pentavalent antimonials have been the treatment of choice for the past 70 years and, due to the appearance of resistant cases, the efficacy of these drugs has come under scrutiny. The present study aimed to investigate the leishmanicidal effect of 17-N-allylamino-17-demethoxygeldanamycin (17-AAG), an HSP90 inhibitor, in vitro. This drug is currently being tested in clinical trials for the treatment of different types of cancer, but its effect on Leishmania infection has never been evaluated. Infected macrophages were treated with 17-AAG and the percentage of infection was determined. Our results show that treatment with 17-AAG resulted in a reduction of up to 98% of the number of macrophages infected with L. amazonensis in a dose- (25-500 nM) and time-dependent manner. Intracellular parasite death occurs independently of the production of NO and superoxide. Then, pro-inflammatory cytokine production was determined. Interestingly, 17-AAG also provokes a reduction in pro-inflammatory cytokine production. Electron microscopy analysis of intracellular parasites revealed the presence of many double membrane vacuoles and myelin figures, suggesting that parasite cell death is related to the autophagic process. To further investigate the mechanism of 17-AAG-dependent parasite death, in the present report we created GFP-ATG8 expressing L. amazonensis. Parasite mutants were treated with 17-AAG and autophagic pathway was monitored. Increase in the number of Leishmania bearing autophagic vacuoles, as well as in the number of vacuoles per cell were observed. Our findings support the idea that 17-AAG-induced parasite death is a mechanism dependent on activation of parasite autophagic pathway.

109 The family M17 Leucyl aminopeptidases from the TriTryps

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Leucyl aminopeptidases (LAPs, EC 3.4.11.1) belong to the M17 family of metallopeptidases and catalyze the cleavage of uncharged amino acid residues, preferentially leucine, from the N-terminus of peptides and proteins. Their natural substrates and role(s) within the cells remain to be established in full, but their housekeeping function in amino acid recycling, degradation of short peptides to single amino acids, and the processing of signal peptides is widely accepted. Additional roles in the regulation of the redox status of the cell, the formation of cataracts and MHC I antigen presentation have been proposed. Bacterial LAPs were shown to be involved in site-directed recombination and transcription regulation (mainly repression). In plants LAPs were reported to have a secondary function as molecular chaperones in response to wound-induced stress. In Plasmodium falciparum, the causative agent of malaria, the M17 LAP is essential for survival in the human host and is hence a good drug target. Given their diverse roles and essential functions in other organisms LAPs are also potential drug targets in the kinetoplastids. We will present structures of the M17 LAPs from T. brucei (2.6 Å resolution), T. cruzi (2.2 Å) and L. major (2.8 Å). The structures, combined with the information gained using biophysical and biochemical techniques, provide valuable insights into the function of these enzymes in kinetoplastids.

110 A model system for investigating VSG switching and coat formation

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The bloodstream form of Trypanosoma brucei relies on high-level expression of the variant surface glycoprotein (VSG), which must form a dense coat on the cell surface of the parasite in order to survive in its mammalian host. Monoallelic expression guarantees that only one VSG of a family of VSGs is expressed at any given time. Active expression occurs from one of approx. 15 telomeric bloodstream form expression sites (BES) present in the genome with other silent VSG genes and pseudogenes also present in the genome. Switching of VSG expression allows the trypanosome to evade the host immune responses and establish a long-term infection in its host. This can occur by different mechanisms involving transcription or recombination switching. We have recently established a model system for studying the processes involved in transcriptional switching. Inducible expression of a second VSG is employed to mimic activation of a second BES. Upon activating expression of the second VSG, trypanosomes respond rapidly by attenuation of the old BES. We have detailed this attenuation process to some extent and found an intriguing reprogramming of the cell. As trypanosomes can generate new mosaic VSGs from genes and pseudogenes present in their genome and the VSG coat is paramount to trypanosome survival, it seems sensible that VSG protein integrity is tested in the course of antigenic variation. We have therefore constructed a set of VSG mutants to study the effect of functionally impaired VSGs during an induced switching process. Our results suggest that VSG protein integrity is indeed monitored and that the cells react in specific but surprisingly different ways.

111 Receptor-cargo complexes form in the cytosol of Leishmania donovani for trafficking to the glycosome.

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Leishmania donovani has a unique microbody organelle called the glycosome that compartmentalizes a variety of metabolic pathways essential for parasite survival. Trafficking and import of newly synthesized proteins depends on cytosolic receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LPEX7), which bind cargo proteins containing a PTS1 (peroxisomal targeting signal) or PTS2 motif, respectively. These receptor-cargo complexes bind to peroxin 14 (LdPEX14) docking complex on the cytosolic face of the glycosomal membrane. This network of protein-protein interactions is required for proper protein targeting. Biochemical analysis of L. donovani cytosolic fractions revealed that LdPEX5 and LPEX7 form heteromeric complexes loaded with PTS1 and PTS2 cargo proteins. To further dissect the molecular process associated with the trafficking and translocation of proteins across the glycosomal membrane we have initiated in vitro studies using the recombinant receptor proteins LdPEX5 and LPEX7 and several model PTS1 and PTS2 proteins. To facilitate these studies we have developed strategies to produce large amounts of the highly hydrophobic LPEX7 in an E. coli expression system. This recombinant receptor protein has been shown to self-oligomerize, and can exist as a monomer, dimer, and tetramer in solution; however, the monomer is sufficient for binding the PTS2 cargos. LPEX7 also forms complexes with LdPEX5, in the presence and absence of PTS1 cargos, which bind the native, glycosomal LdPEX14. This association with LdPEX14 causes the docking protein to undergo a conformational change that drastically increases resistance to protease digestion, most probably via membrane insertion and recruitment of other membrane proteins. The availability of these building blocks will permit studies to re-constitute and characterize the complexes involved in glycosomal cargo protein import.

112 Initiation of VSG switching in Trypanosoma brucei

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The primary mechanism by which Trypanosoma brucei achieves variant surface glycoprotein (VSG) switching is gene conversion by homologous recombination. Although experiments have demonstrated a close association between VSG switching and homologous recombination in T. brucei , the initial stages of the VSG switching mechanism have remained unclear. Here we are investigating the hypothesis that DNA replication stalling occurs upstream of the VSG gene in a region known as the 70 bp repeats and that this precipitates elevated levels of DNA double strand breaks, leading to VSG switching. To test this, we are taking both genetic and biophysical approaches; this poster focuses on the former. We identified four putative replication restart factors and aim to investigate whether they are involved in DNA repair, in particular acting on stalled replication forks, and if they contribute to VSG switching.
Myc-epitope tagging was used for immunofluorescent cellular localisation and knockout mutants are being generated. Analysis of one of these mutants, affecting a RecQ helicase, revealed a growth defect and increased sensitivity to hydroxyurea- and methyl methanesulfonate (MMS) -induced DNA damage. Furthermore, the possible contribution of this RecQ helicase to VSG switching is being investigated using an inducible meganuclease system. Finally, RNAi is being used to examine the function of two kinases, which act as sensors of DNA damage. These tools will allow us to ask about the functions of these factors, test if they display elevated levels of localisation to the VSG 70 bp repeats and whether they contribute to VSG switching.

113 Trypanosoma cruzi RNA Polymerase II is phosphorylated during transcription elongation

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The largest subunit of RNA Polymerase II (Rbp1) has a variable number of heptapeptide (YSPTSPS) repeats at carboxy-terminal domain (CTD). The CTD is highly phosphorylated controlling different steps of transcription events, from initiation, to elongation, splicing, and termination in most eukaryotes. These repeats are absent in trypanosomatids, which control gene expression at the post-transcriptional level. Nevertheless, Trypanosoma brucei Rbp1 is also phosphorylated at the C-terminus but the role of this phosphorylation is unknown. Here we demonstrate that Trypanosoma cruzi Rbp1 is phosphorylated according to the transcriptional status of the RNA Pol II and only the phosphorylated form of TcRbp1 is tightly associated to chromatin fractions. Transcription is arrested in the presence of intercalating agents, such as actinomycin D, without causing dephosphorylation and release of the enzyme from the chromatin. In contrast, in the presence proflavine, hydrogen peroxide, methylmethanesulfonate, heat shock, and nutritional stress, which, differently from actinomycin produce DNA damage in T. cruzi, led to dephosphorylation or RNA Pol II, with dissociation of the transcription machinery from the DNA. However, immunofluorescence analyses reveal that RNA Pol II remains concentrated in nuclear spots, knows to bear the spliced leader RNA genes and possible other transcription initiation sites without transcription progression as seen by bromo-deoxyuridine triphosphate labeling. These findings suggest that RNA Pol II interacts with promoter regions in absence of transcription and that phosphorylation is required for thigh association of the enzyme with the DNA with transcription elongation. Supported by Fapesp and CNPq. *Authors contributed equally to this work.

114 Mitochondrial outer membrane proteome of T. brucei reveals novel factors required to maintain mitochondrial morphology

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The mitochondrial outer membrane (MOM) separates the mitochondria from the cytoplasm, serving both as a barrier and as a gateway. Protein complexes residing in the MOM orchestrate protein and tRNA import, metabolite exchange and lipid metabolism. African trypanosomes are among the earliest diverging eukaryotes that have bona fide mitochondria capable of oxidative phosphorylation. The MOM of T. brucei is essentially unchartered territory. It lacks a canonical TOM-complex and proteins are imported across the MOM using ATOM, which is related to both Tom40 and to the bacterial Omp85-protein family. The beta barrel membrane proteins ATOM, VDAC and Sam50 are the only MOM proteins that have been characterized in T. brucei so far. Using biochemical fractionation and correlated protein abundance-profiling we were able to identify a cluster of 82 candidate proteins that can be localized to the trypanosomal MOM with high confidence Two-thirds of these polypeptides have never been associated with mitochondria before. 40 proteins share homology with proteins of known functions. The function of 42 proteins remains unknown. 11 proteins are essential for the disease-causing bloodstream form of T. brucei and therefore may be exploited as novel drug targets. A comparison with the outer membrane proteome of yeast defines a set of 17 common proteins that are likely present in the MOM of all eukaryotes. Known factors involved in the regulation of mitochondrial morphology are virtually absent in T. brucei . Interestingly, RNAi-mediated ablation of three outer membrane proteins of unknown function resulted in a collapse of the network-like mitochondrion of procyclic cells and therefore directly or indirectly are involved in the regulation of mitochondrial morphology in T. brucei .

115 Chemistry based approach for the identification of a Ufm1 specific processing protease in Leishmania

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Protein ubiquitination plays important functions in many cellular pathways and relies on processing of the Ubiquitin precursor by 3'hydrolase as well as the timely removal of ubiquitin by isopeptidase activities. Ubiquitin-fold modifier 1 (Ufm1) is a recently identified ubiquitin-like protein (Ubl) and like ubiquitin, Ufm1 is synthesized as a precursor that is processed by Ufm1-specific proteases. In a previous study, we demonstrated the existence of the Ufm1 and its conjugation pathway in the trypanosomatid parasite Leishmania donovani . The aim of our study is to identify the specific protease(s) that catalyzes the hydrolysis of the isopeptide linkage to expose the conserved C-terminal glycine of Ufm1. To identify the Ufm1 specific protease, we used an activity-based assay with the suicide inhibitor (6xHis)-Ufm1vinylmethylester (Ufm1G VME) that should irreversibly cross link with the Ufm1 specific protease(s). To prepare Ufm1G VME , a recombinant processed modified Ufm1GC protein was expressed in E.coli that was chemically modified with sodium 2-mercaptoethane sulfonate (MESNa) and finally coupled to glycine-VME. Conversion of Ufm1GC into Ufm1G MESNa and to Ufm1G VME was assessed by Mass spectrometry. We also developed a fluorescence (FRET) based test to assay Ufm1 proteases from parasite lysates. L. donovani amastigote lysates were fractionated over anion exchange columns and FRET positive fractions were fractionated further over cation exchange columns. Double FRET positive fractions were finally used in a pull down assay with Ufm1G VME . Resulting bands were excised from SDS-PAGE gel and subjected to LC/MS/MS analysis using Q-Exactive using an Orbitrap mass spectrometer. Protein hits corresponding to the specific protease will be presented. Ufm1 specific proteases could constitute novel drug target against Leishmaniasis.

116 Evaluation of an aptamer-based assay to detect biomarkers of Trypanosoma cruzi infection in mice treated with Benznidazole

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The blood borne parasite Trypanosoma cruzi (T. cruzi) is the causative agent of Chagas disease. We used an Enzyme Linked Aptamer (ELA) assay to follow the level of a biomarker of T. cruzi infection in blood of mice treated with Benznidazole (Bz) in order to determine if the biomarker level could predict sterile cure. In this assay, Aptamer-L44 developed against T. cruzi excreted/secreted antigens is used as parasite specific ligand. Mice infected with T. cruzi were treated with Bz either during the acute or the chronic phase of the disease. Twenty days after treatment mice were immunosuppressed to promote multiplication of remaining parasites, indicating drug failure. Plasma samples were tested at various times using the AptL44-ELA assay. Our results showed that when mice were treated during the early acute phase, blood parasitemia was controlled and Apt-L44 biomarker level did not increase. Following immunosuppression, parasites appeared in blood and Apt-L44 biomarker level increased. When mice were treated during the chronic phase, no significant difference was observed in the biomarker levels between Bz treated and non-treated animals. Biomarker levels in both groups remained higher compared to non-infected controls. In conclusion, the AptL44-ELA assay can be used to assess the efficacy of a drug to kill blood stage parasites but will not predict sterile cure when the drug is given during the acute phase. This assay is a good indicator of treatment failure when the drug is administered during the chronic phase.

117 Identification of biomarkers of Trypanosoma cruzi infection in blood using aptamers generated against TESA

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Most individuals infected with the blood borne protozoan parasite Trypanosoma cruzi (T. cruzi), causing Chagas disease, are not aware of their chronic infection. This causes a threat to blood supplies because T. cruzi can be transmitted by blood transfusion. Current antibody-based tests have issues of cross reactivity and sensitivity, suggesting a need for an alternative and sensitive non-antibody based assays for T. cruzi diagnostic and blood donors screening. We have generated RNA aptamers (small single stranded nucleic acid molecules) against T. cruzi excreted/secreted antigens (TESA) and used them as specific ligands to develop Enzyme Linked Aptamer (ELA) assays. Aptamer L44 showing high binding affinity and specificity to TESA also reacted specifically with T. cruzi infected mice plasma, in the ELA assay. We used a proteomics based approach to identify the target (biomarker) of aptamer L44. Whole cell extracts of T. cruzi (Tulahuen strain) trypomastigotes, were fractionated using chromatofocusing and the fractions were tested using Apt-L44 ELA assay. Positive and negative fractions were selected and digested with trypsin. The peptides generated were analyzed by 2D nano-LCMS/MS. A comparison of top hits, having zero false positive rates, from ELA positive and negative fractions, resulted in 13 proteins as possible targets of Apt-L44. Of those, 6 were reported to be secreted by some trypanosomes and other parasites. To identify the true target from these candidates, recombinant proteins will be made and assessed for their Apt-L44 binding activity. Additional aptamers will be generated against this target and used to modify the current ELA assay into a quantitative sandwich aptamer-based diagnostic test.

118 Polo-like kinase phosphorylation of bilobe-resident TbCentrin2 facilitates flagellar positioning in Trypanosoma brucei

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In the protist parasite Trypanosoma brucei the single Polo-like kinase (TbPLK) controls the inheritance of a suite of organelles that help position the parasite's single flagellum. These include the basal bodies, the bilobe and the flagellar attachment zone (FAZ). TbCentrin2 was previously shown to be a target for TbPLK in vitro and this is here extended to in vivo studies, highlighting a crucial role for serine 54 in the N-terminal domain. Duplication of the bilobe correlates with the presence of TbPLK and phospho-TbCentrin2, identified using phospho-specific antisera. Mutation of serine 54 leads to slow growth (S54A) or no growth (S54D), the latter suggesting that dephosphorylation is needed to complete bilobe duplication and subsequent downstream events that are necessary for flagellum inheritance.

119 Insights into the architecture and protein interaction network of RNA editing associated complexes in Trypanosoma brucei

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More than 60 proteins co-purify with mitochondrial RNA processing enzymes that include editosomes, the multi-protein complexes responsible for catalyzing mitochondrial RNA editing in Trypanosoma brucei. However, the exact roles and organization of these complexes have yet to be clarified. We hypothesized that characterization of the interaction network of proteins will lead to understanding the essential process of RNA editing in trypanosomatid pathogens and regulatory networks that are connected to editosome and modulate RNA editing and/or processes that depend on RNA editing. Based on available microarray data, our own microarray and high-throughput MS/MS experiments, we reconstructed a high confidence posttranscriptional regulatory network for T. brucei. One of the pathways that we were highly successful to predict was RNA editing; we were able to find several uncharacterized genes that may be involved in RNA editing process and/or mitochondrial RNA processing in T. brucei and contained accessory proteins with known distinct functions such as mitochondrial RNA binding protein 1and 2. To validate the role of some of predicted proteins in RNA editing process, we created transgenic T. brucei cells lines which express tagged proteins. We are currently using these cell lines to purify the tagged candidate proteins to identify the proteins they associate with. Moreover, we used RNAi mediated knockdown and found alterations in their target gRNA/pre-mRNAs. These predictions include assignment of uncharacterized genes to several essential biological processes and pathways that leads us to a better understanding of the biology of trypanosomatids, and also provides new potential targets for treatment of their respective diseases.

120 Negative feedback control mediated by the 3' untranslated region assuring the low expression level of the RNA binding protein TcRBP19 in T. cruzi epimastigotes

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Because of their relevant role in the post-transcriptional regulation of the expression of a multitude of genes, RNA-binding proteins (RBPs) need to be accurately regulated in response to environmental signals in terms of quantity, functionality and localization Transcriptional, post-transcriptional and post-translational steps have all been involved in this tight control. We have previously identified a Trypanosoma cruzi RBP, named TcRBP19, which can barely be detected at the replicative intracellular amastigote stage of the mammalian host. Even though protein coding genes are typically transcribed constitutively in trypanosomes, TcRBP19 protein is undetectable at the epimastigote stage. Here, we show that this protein expression pattern can be explained by the steady-state of its mRNA. Using a T. cruzi reporter gene approach, we could establish a role for the 3' UTR of the tcrbp19 mRNA in transcript down-regulation. In addition, cross-linking followed by immunoprecipitation and in vitro REMSAs confirmed binding of the TcRBP19 protein to its encoding mRNA. Furthermore, we found that forced over-expression of TcRBP19 in T. cruzi epimastigotes decreased the stability of the endogenous tcrbp19 mRNA. These results support a negative feedback control of TcRBP19 abundance, via its cognate 3'UTR, to help maintain it's a very low concentration of TcRBP19 in the epimastigote stage. To our knowledge, this is the first RBP reported in trypanosomatids capable of negatively regulating its own mRNA. The mechanism revealed here adds to our limited but growing number of examples of negative mRNA autoregulation in the control of gene expression.

121 The N-terminal targeting signal of Trypanosome Alternative Oxidase is dispensable for its import into mitochondria

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The trypanosome alternative oxidase (TAO), a nucleus-encoded mitochondrial protein in Trypanosoma brucei that is the sole terminal oxidase in the bloodstage of the parasite, needs to be imported into mitochondria for its function. TAO possesses a predicted N-terminal MTS of twenty-four amino acids; the requirement of this MTS for TAO import has not been experimentally verified. Here we show that deletion of ten amino acids (∆10TAO) from the N-terminus of the protein did not have any affect on the import of TAO whereas the deletion of twenty amino acids (∆20TAO) inhibited its import into mitochondria of the procyclic form in vitro, suggesting that the C-terminal half of the predicted MTS is critical for TAO import into procyclic mitochondria under in vitro conditions. Interestingly, ∆20TAO was imported and processed to its matured form in mitochondria isolated from bloodstream parasites, indicating the requirement of the length of the targeting signal is different in two developmental forms. In contrast to these results we found that TAO was targeted to mitochondria in vivo in both forms of T. brucei after deletion of even 40 amino acids from the N-terminus, suggesting the presence of an internal targeting signal in TAO in addition to the canonical N-terminal MTS. Chimeric proteins consisting of either the first thirty amino acids of TAO (1-30TAO) or a thirty amino acid deletion mutant of TAO (∆30TAO) and dihydrofolate reductase (DHFR) were localized in mitochondria when expressed in the procyclic form. This study reveals that TAO possesses a classical N-terminal MTS along with an internal MTS and both of these signals can function independently.
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122 The Unique Leishmania EIF4E4 N-Terminus is a Target for Multiple Phosphorylation Events and Takes Part in Critical Interactions Required for Translation Initiation

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eIF4E, the cap binding protein, functions during eukaryotic translation initiation as part of the heterotrimeric eIF4F complex with the eIF4G and eIF4A subunits. eIF4F mediates the recognition of the mRNA 5' end and facilitates its binding to the ribosome. In trypanosomatids, two eIF4E homologues (EIF4E3 and EIF4E4) have been shown to participate in eIF4F-like complexes with presumed roles in translation initiation. Both proteins possess unique N-terminal extensions, which have been shown to be targeted by phosphorylation and EIF4E4 participates in a novel interaction with a poly-A binding protein (PABP1) homologue. Here, EIF4E4 phosphorylation was investigated in more detail in Leishmania infantum and seems to be specific for exponentially growing phases of both promastigote and amastigotes life stages. This phosphorylation is observed in overexpressed HA-tagged EIF4E4 and targets multiple serine-proline or threonine-proline residues localized within the protein's N-terminus. EIF4E4 phosphorylation does not require binding to its eIF4G partner (EIF4G3) or the ability to bind mRNA. Mutations in three conserved motifs also mapped to the EIF4E4 N-terminus specifically abolish its unique interaction with PABP1 and no EIF4E4 phosphorylation was observed when both EIF4G3 and PABP1 interactions were eliminated. Attempts to delete both copies of the EIF4E4 gene were unsuccessful, confirming its essential nature, however double knock-outs were recovered in the presence of an episomally encoded wild-type gene. When various mutants were tested using this complementation strategy, loss of PABP1 binding was the single most relevant interaction seen to be required for EIF4E4 function. These results reinforce the unique nature of the translation initiation events in trypanosomatids.

123 Base J Insertion and Function in Leishmania

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β-glucosylhydroxymethyluracil (base J) is a modified DNA base thus far found only in Euglenozoa. Base J synthesis requires two enzymes (JBP1 and JBP2) that catalyze hydroxylation of thymine, and the resultant HOMeU is modified by a putative glucosyltransferase. We have previously shown that knock-out of JBP2 in Leishmania caused gradual loss of J, transcriptional read-through at convergent strand-switch regions (cSSRs) that contain internal J (iJ), as well as false-starts at transcription initiation sites. Bromodeoxyuridine (BrdU) treatment of JBP2KO parasites caused further reduction in iJ levels, and eventual death of the cells. We have now used RNA-seq and Nanostring technology to quantify mRNA levels in BrdU-treated WT L.□tarentolae and a new JPB2KO line, revealing consistent and substantial changes in expression of several genes near iJ sites. These results suggest that loss of iJ induces de-repression of genes deleterious to cell growth, and/or down-regulation of essential genes (due to accumulation of antisense RNA at cSSRs). To investigate the signals responsible for J insertion, we cloned several J-containing sequences into plasmids and grew them episomally in L.□tarentolae. Plasmids containing cSSR-25.2 sequence or the telomeric hexamer sequence ([GGGTTA]10) accumulated J when grown in WT but not JBP2KO cells, while those containing the atypical cSSR-28.2 (which lacks iJ in WT cells) accumulated no detectable J. SMRT sequencing of the J-containing plasmids revealed that most J sites occurred in pairs at 13-nt intervals on opposite DNA strands. Thus, we hypothesize that JBP2 recognizes the signal for de novo J insertion, while JBP1 is responsible for J maintenance (following DNA replication) by recognizing this J and inserting a new J downstream on the opposite strand.

124 Comparative transcriptomics of Trypanosoma cruzi primary and secondary amastigogenesis

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Trypanosoma cruzi is the protozoan that causes Chagas disease, illness that affects about 15 million people in the American continent. This parasite has a biphasic life cycle in which four cellular forms alternate between the insect vector (epimastigotes and metacyclic trypomastigotes) and the mammalian host (amastigotes and bloodstream trypomastigotes), being that 4 differentiation events are known: epimastigogenesis, metacyclogenesis, amastigogenesis and trypomastigogenesis. The amastigogenesis naturally accurs when trypomastigotes penetrate mammal cells and differentiate to amastigotes. Depending on the source of trypomastigotes, the amastigogenesis can be classified as primary or secondary if started from metacyclic or blood trypomastigotes, respectively. The present work aims to analyze the transcriptome of this parasite during in vitro primary and secondary amastigogenesis using RNA-Seq technology. Metacyclic trypomastigotes were obtained by in vitro metacyclogenesis in TAU3AAG medium whereas cell derived trypomastigotes were recovered from the supernatant of infected Vero cells cultures. Both trypomastigotes forms where purified by ion exchange chromatography in DEAE-cellulose columns and the amastigogenesis was induced by exposing the parasites to high glucose DMEM medium at pH 5. As the amastigogeneses have differentially kinetic properties, total RNA samples were obtained at different time time points: 0, 12, 24, 36, 48 and 72 hours for primary amastigogenesis and 0, 2, 6, 12, 24 and 48 hours for secondary amastigogenesis. Samples were analyzed by massive parallel sequencing (RNA-Seq) with the SOLiD4 platform. Currently we are analyzing the 36 libraries produced (triplicate experiments) in search for differentially expressed genes and patterns of gene expression between primary and secondary amastigogenesis. These data constitute the first global assessment of T. cruzi transcriptional program during amastigogenesis.

125 Spatiotemporal Localization of Mitochondrial DNA Polymerases in Trypanosoma brucei.

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Trypanosome mitochondrial DNA, known as kinetoplast DNA (kDNA), is organized into a single disk shaped nucleoid that is a catenated network of minicircles and maxicircles. kDNA is essential for survival and requires a topoisomerase-mediated release and reattachment mechanism for minicircle replication, resulting in spatial and temporal separation of replication events. Additionally, trypanosomes contain multiple proteins with non-redundant roles in kDNA replication, and discrete localization patterns around the kDNA network, mainly in the kinetoflagellar zone and antipodal sites. The spatiotemporal dynamics of kDNA replication proteins and how they coordinate their functions during replication are not well understood. We have been investigating the cell-cycle dependent localization of three essential mitochondrial DNA polymerases (TbPOLIB, IC and ID), to establish protein markers for different stages of kDNA replication. Using Trypanosoma brucei as a model for mitochondrial DNA replication dynamics, we identified a dynamic localization pattern for POLID that involves redistribution from the mitochondrial matrix to the antipodal sites during early kDNA S phase. POLIC also accumulated as foci at the antipodal sites only at early replication stages, and colocalized with POLID and replicating minicircles. Here, we examine the spatiotemporal localization of POLIB and demonstrate that, unlike POLIC and POLID, POLIB signal is detected near the kDNA disk at all cell cycle stages, but is not antipodal. The localization pattern does change during kDNA replication with POLIB signal becoming more elongated at the later stages. Lastly, POLIB RNAi disturbs the localization pattern of POLIC, indicating that localization of one polymerase could depend on the localization of another. Therefore, we are also investigating the colocalization of POLIB and the other essential mitochondrial DNA polymerases.

126 Overexpression of a Trypanosoma brucei RNA-Binding Protein in vitro Promotes Metacyclogenesis

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Recently our laboratory developed an in vitro system recapitulating the Trypanosoma brucei tsetse fly life-cycle stages through conditional overexpression of the RNA binding protein 6 (RBP6). Cultured procyclic cells differentiate into epimastigotes within 24 hours and after about 7-10 days, into infectious metacyclics. We now have used stage-specific markers, such as BARP, calflagin, trypanosome alternative oxidase, and the clathrin heavy chain, to follow epimastigote and metacyclic accumulation. To understand the changes in gene expression following RBP6 induction we analyzed mRNA abundance after 24 hours of induction by mRNA-Seq. By immunofluorescence the majority of cells express RBP6 at this time point. Using 2-fold as the threshold we observed 121 upregulated and 59 downregulated transcripts. Interestingly, the transcripts of two subtelomeric gene families coding for small proteins of unknown function and an atypical Variable Surface Glycoprotein gene were significantly upregulated. Analysis and validation of the RNA-Seq data will be presented. Next, we asked whether differentiation requires continuous RBP6 expression. Exposure of cells to doxycyline for various periods of time revealed that lengthening the time of RBP6 expression from 1 to 10 days increases the accumulation of metacyclics, thus suggesting that at each time point only a certain proportion of cells are committed to differentiation and/or perhaps RBP6 is required at later stages after the initial induction. Lastly, to improve the yield of metacyclics we varied the culture conditions and found that increasing the glucose concentration was the most important parameter leading to ~50% metacyclics after 7 days of induction. Varying the serum concentration, the availability of CO 2 , decreasing the temperature or starving cells prior to induction did not significantly affect metacyclic yield.

127 Haptoglobin-hemoglobin receptor-independent uptake of trypanosome lytic factor 2

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Trypanosome lytic factor 2 (TLF2) is a 1,000 kDa HDL-associated immunocomplex containing the pore-forming protein apolipoprotein L-I as well as haptoglobin related protein (Hpr) and an IgM antibody. TLF2, the main lytic factor in human serum, is lytic on African trypanosomes lacking the haptoglobin-hemoglobin receptor, which binds Hpr-hemoglobin and is the primary uptake pathway for TLF1. Among primates expressing TLFs, TLF2 has only been found in humans. The major uptake route for TLF2 remains unknown. Sequencing shows the TLF2 IgM is polyclonal (k and l chains) and belongs to the VH3 family, most common among human polyreactive antibodies. As we have found that the IgM is polyreactive, recognizing dsDNA, lipopolysaccharide, and Hpr (1:100 serum equivalent), we conclude TLF2 contains a "natural IgM antibody", which is characteristically polyreactive against multiple self and foreign antigens but is nonpathogenic. The interaction with Hpr appears necessary for the formation of the TLF2 complex from IgM and pre-beta TLF1. Such "natural antibodies" are encoded by germline variable genes that have not undergone considerable somatic mutation and have a broad range of binding avidities (5x10 -3 -to 5x10 -11 M). Most natural antibodies belong to the IgM isotype and circulate in serum at low titers. Variant surface glycoproteins (VSGs), which form the coat of African trypanosomes, are recognized by polyreactive natural antibodies and therefore could be recognized by TLF2. We hypothesize that the IgM plays a role in uptake of TLF2 via interaction with VSG and clearance from the parasite surface. Using a blot overlay assay, the VSG-IgM interaction is examined alongside other TLF2 IgM targets to elucidate the role of VSG in the TLF2-mediated trypanolytic activity of human serum.

128 Control of mitochondrial shape in Trypanosoma brucei by TbLOK1, a novel outer membrane protein

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By screening an RNAi library for loss of kinetoplast DNA (kDNA), we discovered an uncharacterized T. brucei protein that we call TbLOK1. We found that TbLOK1 is an integral membrane protein located in discrete patches along the outer mitochondrial membrane. Following depletion of TbLOK1, the highly branched mitochondrial structure characteristic of procyclic trypanosomes collapsed into a single tubule, similar to the streamlined organelle seen in bloodstream forms. These changes in mitochondrial structure were accompanied by defects in mitochondrial membrane potential, respiration, and transcription. At later times following TbLOK1 knockdown, a more drastic alteration in mitochondrial shape occurred and kDNA was lost. Since knockdown of known kDNA replication and transcription proteins led to a much more rapid loss of kDNA, with little or no effect on mitochondrial structure, we conclude that TbLOK1 does not play a direct role in kDNA maintenance. We instead speculate that TbLOK1 plays an critical role in maintaining the branched architecture of the mitochondrion in procyclic cells, and that this morphology is crucial for proper organelle function. Intriguingly, TbLOK1 is downregulated in bloodstream trypanosomes, raising the possibility that this novel protein might directly control mitochondrial shape in the different life cycle stages of T. brucei.

129 A Novel Method for Mitochondrial RNA Knockdown of ATPase Subunit A6 mRNA in Trypanosoma brucei

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Trypanosomes possess a unique mitochondrial genome called the kinetoplast (kDNA), many of which produce pre-mRNAs that must undergo guide RNA (gRNA)-mediated RNA editing. One example of an extensively edited kDNA gene is ATPase subunit A6. A6 forms the mitochondrial proton half channel, of the F0F1 ATPase, and is critical for mitochondrial membrane potential. While procyclic form (PF) cells undergo active oxidative phosphorylation, the mammalian infectious blood stream form (BF) cells do not. Instead, BF cells utilize substrate level phosphorylation to produce ATP, a portion of which is hydrolyzed by the F0F1 ATPase to pump protons into the inner membrane space. To better understand the mechanism and function of mitochondrial RNAs in trypanosomes, it is critical to develop reverse genetic approaches allowing for in vivo analysis, which are unavailable in the mitochondria. Artificial site-specific RNA endonucleases (ASREs) combine a sequence specific single stranded RNA (ssRNA) recognition domain with a RNA endonuclease domain. The ssRNA binding domain of the ASRE can be reprogrammed to recognize unique eight-nucleotide sequences, thus we can design ASREs to cleave any target RNA. Utilizing a nuclear-encoded ASRE containing a mitochondrial localization signal, we have shown knock-down of a canonical mitochondrial mRNA. Expression of an A6-targeted ASRE in PF trypanosomes shows an approximately fifty percent reduction in A6 mRNA levels and a time-dependent decrease in mitochondrial membrane potential. ASRE expression results in a significant growth decrease in PF trypanosomes, however, expression in BF trypanosomes shows no significant growth effect. Knock-down of A6 mRNA allows for the first in vivo analysis of an edited mitochondrial mRNA. This method will provide critical insight into mitochondrial RNA biology in trypanosomes.

130 Validating Trypanosoma cruzi spermidine synthase as a target for rational drug design against Chagas disease: an integrated structural biology and molecular genetics approach.

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Trypanosoma cruzi causes Chagas disease and infects an estimated ten million people in the Americas, including 300,000 in the United States. Existing treatments are highly toxic and have uncertain efficacy against the chronic stage of the disease, which frequently involves fatal cardiomyopathy and severe gastrointestinal pathology. Polyamine biosynthesis is a promising target for novel drug development in T. cruzi, as it comprises the target in the related parasite Trypanosoma brucei of the African sleeping sickness drug Nifurtimox (difluoromethylornithine, DFMO). Although the molecular target of DFMO is absent in T. cruzi, a promising alternative is spermidine synthase (TcSpdSyn), catalyzing the final step in T. cruzi polyamine biosynthesis. We have determined X-ray crystal structures of TcSpdSyn at 2.3Å and 2.1Å resolution, alone and in complex with the reaction product methylthioadenosine. The structures reveal an active site architecture with binding and catalytic residues closely corresponding to the archetypal configuration of human and Plasmodium falciparum SpdSyn, suggesting that SpdSyn inhibitors developed as anticancer and antimalarial drugs or lead compounds could be readily adapted to target T. cruzi. A key distinct structural feature of TcSpdSyn is the exceptionally high temperature factors of two catalytically important tyrosine residues and adjacent loops well-ordered in the human enzyme, indicating structural disorder that might be exploited to selectively target TcSpdSyn. To biologically validate the enzyme as a drug target, we are undertaking the deletion of each of the four TcSpdSyn genomic loci. Should TcSpdSyn be positively established as a drug target, we are poised to initiate the structure-guided design of highly selective SpdSyn inhibitors to serve as leads for the development of safe and effective therapies for Chagas disease.

Poster Session: B

04/23/2013

132 Evaluation of the Apoptotic-like Activity of Ruthenium-Clotrimazole Compounds against Leishmania major

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Leishmaniasis caused by the parasite L. major affects millions of people worldwide, about 12 million cases in 88 countries. Current available drugs present high toxicity and/or low efficacy. In order to understand the actual mechanism of action of the Ruthenium-Clotrimazole compounds previously tested in our laboratory; we evaluated the apoptolic-like activity of the compounds in the parasite. First, we treated the parasites for 24 hrs, followed by Alexa Fluor 488 Annexin V and Propidium Iodide staining. The stained cells were then analyzed by flow cytometry using Cytomic FC 500. Additionally, we performed a JC-1 Mitochondrial Membrane potential assay to analyze if the Ru-CTZ compounds kill the parasite by lost of mitochondrial membrane potential. The parasites were treated for 6 hours, followed by the JC-1 staining and analyzed by flow cytometry as well. AM162 at 1.87 µM, showed 71.5%, and AM160 at 20 µM, 59.1% of treated cells presented early/late apoptosis. In comparison with the negative control with only 1.6% and the positive control, Hydrogen Peroxide at 200 µM with 56.2%. Moreover, in the JC-1 assay, AM162 at 1µM with 94.8% and AM160 at 1 µM with 96% representing the mitochondrial membrane alterations in the parasites treated, in comparison with the negative control with only 38.4%. In conclusion, the compounds are able to induce an apoptosis-like mechanism by the flipping of Phosphatidylserine on the plasma membrane in L. major promastigotes. In addition, the compounds are able to disrupt the mitochondria membrane potential of the parasites producing apoptosis like effect. Based on these experiments, and preliminary data, these compounds represent excellent leads for the development of new chemotherapeutic agents to treat leishmaniasis.

133 Identification of transmission stage-specific gene regulators in Trypanosoma brucei via a genome-wide RNAi selection approach

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African trypanosomes, responsible for sleeping sickness, undergo developmental transitions during their life cycle, including differentiation of proliferative slender parasites to non-proliferative, transmissible, stumpy forms in the mammalian host. During this transition, a small subset of genes is upregulated, one example being the ESAG9 gene family, whose expression is highly elevated in stumpy forms. In trypanosomes, almost all genes are regulated post transcriptionally, but the details of how this regulation is carried out remains still unknown. A bifunctional short (34nt) regulatory RNA element present in the 3'UTR of one highly conserved ESAG9 gene has been identified, which is responsible for gene silencing in slender forms and gene activation in stumpy forms. The use of this small specific control element as a ligand provides a powerful tool to identify proteins that are able to bind it and silence the expression of the associated ESAG9 gene 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 Neomycin selection, this selected parasites where ESAG9 3'UTR mediated gene silencing in slender forms was reduced, generating increased neomoycin expression. This identified several genes, including one encoding for an RNA binding protein, that are potentially involved in the ESAG9 regulation pathway. These are currently being studied to characterise their specific role in the regulation of the slender to stumpy transition. These results will help to better understand the mechanistic basis of transmission stage gene expression, generating insight into the mechanisms that contribute to optimise parasite spread.

134 A Single Amino Acid Substitution in the Trypanosoma brucei gambiense Haptoglobin-Hemoglobin Receptor Abolishes TLF-1 Binding

DeJesus, Eric; Hajduk, Stephen

Critical to human innate immunity against African trypanosomes is a minor subclass of human high-density lipoproteins, termed Trypanosome Lytic Factor-1 (TLF-1). This primate specific molecule binds to a haptoglobin-hemoglobin receptor (HpHbR) on the surface of susceptible trypanosomes initiating a lytic pathway. Group 1 Trypanosoma brucei gambiense causes human African Trypanosomiasis (HAT) escaping TLF-1 killing due to reduced uptake. Previously, we found that group 1 T. b. gambiense HpHbR (TbgHpHbR) mRNA levels were greatly reduced and the gene contained substitutions within the open reading frame. Here we show that a single, highly conserved amino acid in the TbgHpHbR ablates high affinity TLF-1 binding and subsequent endocytosis thus evading TLF-1 killing. In addition, we show that over-expression of TbgHpHbR failed to rescue TLF-1 susceptibility. These findings suggest that the single substitution present in the TbgHpHbR directly contributes to the reduced uptake and resistance to TLF-1 seen in these important human pathogens.

135 Trypanosoma brucei Vacuolar Transporter Chaperone 4 (TbVTC4) is an acidocalcisomal polyphosphate kinase required for in vivo infection

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Polyphosphate (polyP) is an anionic polymer of orthophosphate groups linked by high energy bonds that typically accumulates in acidic, calcium-rich organelles known as acidocalcisomes. PolyP synthesis in eukaryotes was unclear until it was demonstrated that the protein named vacuolar transporter chaperone 4 (Vtc4p) is a long chain polyP kinase that localizes to the yeast vacuole. Here, we report that the Vtc4 ortholog of Trypanosoma brucei (TbVTC4) encodes, in contrast, a short chain polyP kinase that localizes to acidocalcisomes. The subcellular localization of TbVTC4 was demonstrated by fluorescence and electron microscopy of cell lines expressing TbVTC4 in its endogenous locus fused to an epitope tag and by purified polyclonal antibodies against TbVTC4. Recombinant TbVTC4 was expressed in bacteria and polyP kinase activity was assayed in vitro. The in vitro growth of conditional knockout bloodstream form (BSF) trypanosomes (TbVTC4 cKO) was significantly affected relative to the parental cell line. This mutant had reduced polyP kinase activity and short chain polyP content, and was considerably less virulent in mice. The wild type phenotype was recovered when an ectopic copy of TbVTC4 gene was expressed in the presence of doxycycline. The mutant also exhibited a defect in volume recovery under osmotic stress conditions in vitro, underscoring the relevance of polyP in osmoregulation. Since VTC4 is absent in vertebrates, we tested a number of kinase inhibitors on the enzyme activity and BSF trypanosomes growth in vitro and our results provide proof-of-concept that inhibitors of this enzyme may facilitate further drug development and parasite control.

136 A mitogen-activated protein kinase is required for basal body duplication and segregation and cytokinesis in Trypanosoma brucei

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Mitogen-activated protein kinase (MAPK) modules are evolutionarily conserved three-kinase cascades that function in response to the environment and stress and serve crucial roles in intracellular signal transduction in eukaryotes. A MAPK cascade is required for cytokinesis in plants, but the involvement of a MAPK cascade in regulating the cytokinesis of other organisms has not been documented. Here, we report that a MAPK homolog (TbMAPK4) from Trypanosoma brucei , a parasitic protozoan and the causative agent of human sleeping sickness, is required for basal body duplication/segregation and cytokinesis and appears to play distinct roles in the two life cycle forms of T. brucei . TbMAPK4 is distributed throughout the cytosol in the procyclic form, but is localized in both the cytosol and the nucleus in the bloodstream form. In the cytoskeleton of the trypanosome cells, TbMAPK4 associates primarily with the flagellum in the procyclic form, whereas it is enriched along the flagellum attachment zone in the bloodstream form. RNAi of TbMAPK4 resulted in moderate growth inhibition in the procyclic form, but led to severe growth defect and rapid cell death in the bloodstream form. Despite the distinct growth defect in the two forms, however, TbMAPK4 deficiency compromised basal body duplication and segregation in both life cycle forms. Finally, we found that TbMAPK4 is required for the completion of cytokinesis in the procyclic form, but is essential for the initiation of cytokinesis in the bloodstream form. Altogether, our results demonstrate the involvement of a MAPK-mediated pathway in promoting basal body duplication and segregation in trypanosomes and identify distinct roles of TbMAPK4 in cytokinesis between the two life cycle forms.

137 Trypanosomes possess a distinct XPB helicase for nucleotide excision repair that functions independently of a TFIIH complex

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Conserved from yeast to humans, TFIIH is an essential factor for transcription initiation of RNA polymerase II and for nucleotide excision repair (NER). TFIIH consists of a core of seven subunits including the DNA helicases Xeroderma pigmentosum B (XPB) and XPD, and of a kinase subcomplex. Trypanosoma brucei TFIIH comprises a full core complex and two additional subunits which likely represent subunits of the basal transcription factor TFIIE; a kinase was not detected. Analogously to the yeast/human systems, T. brucei TFIIH was shown to be indispensable for RNA polymerase II transcription of the spliced leader (SL) RNA genes. Trypanosomatid organisms, however, possess two highly divergent paralogs of XPB but only the larger one, XPB-L, was consistently identified in TFIIH purifications whereas the shorter one, termed XPB-R (R for repair), did not co-purify. Analyzing the functional significance of XPB-R, we found out that XPB-R is not essential in cultured procyclics. XPB-R-/- knockout cells exhibited a consistent slow-growth phenotype over many generations. As assessed by in vitro transcription assays and nascent RNA analysis, XPB-R depletion/removal had no effect on gene transcription. Correspondingly, chromatin immunoprecipitation did not detect XPB-R occupancy at SL RNA genes. These results ruled a transcriptional function of XPB-R out. Conversely, wild-type and XPB-R-/- cells differed significantly in their tolerance to UV light- and cisplatine-induced DNA damage that require NER. Since this difference was not observed in treatments with the DNA mismatch repair-inducing agent methyl methanesulfonate, these findings identified XPB-R as a helicase dedicated to NER. Since XPB-R functions independently of a TFIIH complex and since several other early-diverged eukaryotes contain similarly divergent XPB paralogs, it appears that the dual function of TFIIH in transcription and DNA repair is a synapomorphic trait of fungi and higher eukaryotes.

138 Regulation of mitochondrial function and virulence by a scaffold protein in Leishmania

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RACK1, a WD-40 family scaffold protein, regulates various cellular activities including signaling and translation. Prior studies of the Leishmania RACK1 ortholog, LACK, indicate that threshold levels of LACK (expressed from a minimum of two LACK gene copies) are required for robust survival at host temperatures (~35°C) in vitro and virulence in vivo . To further investigate how LACK promotes parasite fitness in the mammalian host, we compared the proteomes of virulent LACK -two copy (LACK/LACK) with attenuated LACK -single copy (LACK/-) L. major strains that had been incubated at 35°C. This approach identified several proteins, including subunits of the mitochondrial cytochrome c oxidase complex, whose levels were differentially modulated in LACK/LACK versus LACK/- L. major . Using immunoblotting, we confirmed that cytochrome c oxidase subunits IV (LmCOX4) and VI (LmCOX6) were significantly decreased in LACK/- compared to LACK/LACK L. major promastigotes cultured at 35°C, but not at 27°C. Importantly, LmCOX4 and LmCOX6 levels were also substantially decreased in lesion amastigotes of LACK/- L. major . To determine the physiological impact of LmCOX4 deficiency, as observed in the LACK/- line, we assayed for cytochrome c oxidase activity, mitochondrial membrane potential, and ATP generation. These experiments confirmed that, at 35°C, COX activity is significantly decreased in LACK/- compared to LACK/LACK L. major . Likewise, mitochondrial membrane potential and ATP generation are also reduced in LACK/- L. major under these conditions. Currently, experiments are underway to further elucidate molecular mechanisms that underlie the requirement for LACK in maintaining parasite mitochondrial fitness under mammalian conditions. To our knowledge, these studies are the first to demonstrate a role for RACK1 proteins in regulating mitochondrial function.

139 ZC3H32 is an essential zinc finger protein that can destabilise RNA

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Zinc finger proteins of the C3H type are often RNA binding proteins, which can either stabilise or destabilise their target RNAs. An in-silico screen by Kramer et al. (1) has indentified 48 C3H-type zinc finger proteins in Trypanosoma brucei. ZC3H32 first caught our attention when a yeast-two-hybrid screen suggested it as an interaction partner of MKT1. This interaction was confirmed by Co-Immunoprecipitation. MKT1 is thought to stabilise RNA by binding to PBP1, which in turn binds to the poly-A binding protein. ZC3H32 localizes to the cytoplasm, but, unlike MKT1, it is not associated with polyribosomes. Also, artificial tethering of ZC3H32 to a reporter RNA leads to its degradation, which points towards a destabilising role. This is surprising, as artificial tethering of MKT1 shows the opposite effect. However, the yeast-two-hybrid data also suggests NOT2, a member of the CAF1-NOT deadenylase complex as a putative interaction partner of MKT1. RNAi against ZC3H32 in the bloodstream form leads to growth arrest and also causes changes in the abundance of many RNAs. The SILAC proteomic analysis by Urbaniak et al. (2) showed that the protein level of ZC3H32 is elevated in the bloodstream form, as compared to the procyclic form. Overexpression of ZC3H32 in procyclics does not affect the trypanosomes' growth; however, it does influence the levels of many RNAs. Our data suggests that ZC3H32 is a destabilising RNA binding protein, which plays an important role in the bloodstream form. 1. Kramer S, Kimblin N, Carrington M. 2010. BMC Genomics 11: 283 2. Urbaniak MD, Guther MLS, Ferguson MAJ. 2012. PLoS ONE 7: e36619

140 Trypanosoma brucei contains two Asf1 forms with distinct cellular localization and histone chaperone function

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The Anti-silencing function protein 1 (Asf1) is a chaperone that forms a complex with the histone H3 and H4, facilitating the dimer deposition and removal from the chromatin. Asf1-histone complexes participate in the histone translation, and in several processes that involve chromatin remodeling, such as DNA replication, transcription, and repair. Most eukaryotes display two different copies of Asf1 but their specific functions are still unknown. Kinetoplastida also have two different Asf1, Asf1A (19 kDa) and Asf1B (24 kDa) and previous work have indicated that, different from other organisms, both are essential for Trypanosoma brucei (Li et al, J Cell Sci 120: 3883). Phylogenetic analyses show that these two Asf1 genes are more divergent than the two forms of other organisms, suggesting that they might have unique roles in Trypanosomatids. Indeed, T. brucei Asf1B knockdown arrests procyclics in the S-G2 transition, while Asf1A knockdown results in the accumulation of cells in S phase of the cell cycle. Monospecific antibodies to each Asf1 reveal that Asf1A is restricted to the cytosol, while Asf1B is predominantly in the cell nucleus. Identical results were obtained by overexpression of untagged or Myc-tagged protein in both N and C-terminus, or Myc-tagging the endogenous locus, indicating a distinct function for the trypanosome proteins. In fact, only the overexpression of the cytosolic Asf1 causes an increase in the level of histone H3 and H4 in the cell, indicating that it is related to the histone synthesis. In contrast, only the overexpression of the nuclear Asf1 causes a poor cell cycle arrest in parasites exposed to genotoxic agents, prompting to a function in the control of chromatin assembly. In conclusion, these findings denote a unique complexity of the histone metabolism in Kinetoplastida. FAPESP and CNPq

141 Characterization of the trypanosome PRP19 complex involved in cis and trans splicing of nuclear pre-mRNA

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In cis splicing of nuclear pre-mRNA (intron removal), the PRP19 complex plays an essential role as part of the activated spliceosome that consists of the U2, U5 and U6 snRNPs and many non-snRNP proteins. While trypanosomatid parasites contain very few intron-disrupted genes, all nuclear pre-mRNA is processed by spliced leader (SL) trans splicing in which the 5/-terminal sequence of the SL RNA is fused to the 5/ end of each mRNA. Previously, PRP19 silencing was shown to block the first splicing step and to affect methylation of the SL RNA cap4 structure that is important to the trans splicing process (Tkacz et al., 2010, JBC 285:27982). To characterize the PRP19 complex in Trypanosoma brucei, we fused the composite PTP tag C-terminally to PRP19 in one procyclic cell line and to CDC5, another subunit of the complex, in a second cell line. Tandem affinity purification of these tagged proteins combined with sucrose gradient sedimentation revealed the same co-sedimenting complex of seven subunits comprising PRP19, CDC5, PRP17, PRP45, PRP46, SNF27, and PPIL1. This composition differs from both the PRP19 complex in humans and the corresponding nineteen complex in yeast. An RNA analysis showed that U2, U5, and U6 snRNA but not U1 or U4 snRNA precipitated with PRP19 and CDC5 confirming that the PRP19 complex, like its yeast and human counterparts, is part of the activated spliceosome. Among the seven subunits, SNF27 was the least conserved and its identification primarily based on a short, highly conserved sequence motif. Nevertheless, SNF27 silencing abrogated RNA splicing and cap4 methylation demonstrating that PRP19 complex function and SL RNA cap4 formation are intimately linked.

142 Mitochondrial production of acetate is essential for viability of the Trypanosoma brucei bloodstream forms

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During its life cycle, Trypanosoma brucei alternates between a mammalian host (bloodstream forms - BSF) and insect vector, the tsetse fly (procyclic form - PCF). Using PCF as a model, we showed that acetate produced in the mitochondrion from glucose and threonine is essential to feed lipid biosynthesis through a new metabolic pathway called "acetate shuttle". In this shuttle, acetate produced in the mitochondrion from acetyl-CoA by two different enzymes, i.e. acetate:succinate CoA-transferase (ASCT) and acetyl-CoA thioesterase (ACH), is exported in the cytosol and converted back to acetyl-CoA (the precursor for fatty acid biosynthesis) by the cytosolic AMP-dependent acetyl-CoA synthetase (AceCS) (ref). In addition, acetate production is important for ATP production in the mitochondrion (ref). In contrast to PCF, the acetyl-CoA/acetate metabolism of the BSF has been largely underestimated. Indeed, we observed that BSF produce acetate from glucose although this pathway was considered non-existent in these forms. Here, we addressed the function of key enzymes involved in this pathway in BSF. We determined the localization of the pyruvate dehydrogenase (PDH), the threonine dehydrogenase (TDH) and the AceCS. We also investigated the role of these enzymes in acetate production and lipid biosynthesis, by the production of a number of single and double RNAi and/or KO mutants in BSF. We clearly demonstrated that acetate production is also essential for BSF. Thus, we propose that acetate production is a key pathway for both energy production and biosynthetic purposes in BSF

143 Pharmacological assessment of the Leishmania casein kinase protein family reveals CK1.2 as important drug target with essential functions in intracellular parasite survival

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The Leishmania genome encodes for six CK1 isoforms, including LmjF35.1010 (CK1.2), which represents the most conserved kinase among Leishmania species and is the only isoform previously identified in published proteomic studies. LmCK1.2 is an ecto-kinase, released from promastigotes in culture and present in parasite exosomes. Together these findings suggest that CK1.2 is likely the most relevant member of this protein kinase family in Leishmania, with crucial functions in parasite biology and infection. Here, we applied a pharmacological approach using the CK1-specific inhibitor D4476 to assess the importance of CK1.2 for Leishmania extra- and intracellular survival. Using a CK1.2-specific antibody, we first showed constitutive kinase expression in promastigotes and axenic amastigotes. We next revealed kinase activity of purified recombinant and transgenic CK1.2 from both stages, which was efficiently inhibited by D4476. Significantly, D4476 is toxic for both parasite stages, suggesting essential functions for CK1 kinases in parasite viability in vitro. Similarly, inhibitor treatment of macrophages hosting virulent L. donovani amastigotes substantially decreased intracellular parasite burden, thus validating CK1 kinases as drug targets with essential functions in survival of the pathogenic amastigote stage in situ. Using recombinant CK1.2 we screened kinase-biased chemical libraries representing over 5,000 compounds and identified CK1.2 inhibitors with IC50 values between 0.07 and 9.5 µM that were subsequently validated on extra- and intracellular parasites. Our screening identified novel hit compounds representing different chemical scaffolds with potent anti-leishmanial activity down to 60 nM. Structure Activity Relationship (SAR) analyses are currently performed for selected compounds to increase potency and develop leads for future structure-guided drug design.

144 Polyphosphate polymerase in Leishmania parasites

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Polyphosphate (polyP), a multifunctional and ubiquitous polymer, was found to be involved in several cellular processes, such as osmoregulation, adaptation to stress and virulence of pathogens. Therefore, we decided to investigate the importance of polyP in the protozoan parasite Leishmania. This pathogen is the causative agent of the human parasitic disease leishmaniasis, which is estimated to affect 12 million people in 88 countries. Recently, the vacuolar transporter chaperon 4 (Vtc4) has been identified as a polyP polymerase in S. cerevisiae. In yeast, Vtc4 was shown to be part of a protein complex that is mainly localized in the vacuolar membrane. The major polyP pools of yeast are found in vacuoles, while Trypanosomatids store large amounts of short and long chain polyP in calcium-rich acidic organelles known as acidocalcisomes. Homologues of the Vtc proteins were found in trypanosomatid parasites but not in humans. During our study we observed that cellular polyP levels fluctuate during the leishmania life cycle, being highest during the proliferating logarithmic phase. After identifying vtc homologues in Leishmania major, we created vtc4 knock-out parasites by homologous recombination. The deletion of vtc4 resulted in polyP deficient L. major, indicating a polyP synthesizing activity of the enzyme. Besides lacking polyP, the promastigotes did not show major defects under optimal growth conditions. However, absence of Vtc4 protein and polyP seemed to impair parasite resistance to stress as well as parasite virulence in vitro and in vivo.

145 Functional characterization of Mucin-Associated Surface Protein (MASP) in the human parasite Trypanosoma cruzi

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MASPs are members of a multigenic family of the T. cruzi CL Brener genome. Highly conserved N- and C-terminal domains and a hypervariable central region, characterize MASP that is predominantly expressed in the infective trypomastigote form. We hypothesized that members of the T. cruzi MASP protein family play a major role in the interaction of the parasite with the host cell. To investigate a putative role for T. cruzi MASP, we used MASP as bait against the human proteome using a high-throughput platform for identifying protein-protein interactions. Yeast two-hybrid screens identified human SNAPIN as a major MASP interacting protein. SNAPIN is known to be implicated in calcium-dependent exocytosis. The interaction was further validated using co-Affinity Purification and pull-down assays. Immunofluorescence assays showed SNAPIN is recruited to the parasite surface during invasion and co-localized with the host late endosomes and lysosomes. SNAPIN depletion and deletion in the host cells significantly inhibited T. cruzi invasion. The parasites invading snapin-/- MEF cells did not recruit host lysosomes as efficiently as the ones in wild-type cells. We also showed that SNAPIN was translocated to the plasma membrane upon calcium influx induced by a calcium ionophore, resulting in the exposure of the luminal domain of SNAPIN to the extracelluar space. Leishmania tarentolae transgenic strains expressing two different MASP proteins triggered a transient intracellular calcium flux in HeLa cells. It is likely that T. cruzi MASP plays a role in wounding the host plasma membrane, which in turn elicits a transient intracellular calcium flux and leads to the translocation of lysosome-associated SNAPIN to the plasma membrane. SNAPIN, through its exposed luminal domain, would then provide an anchor for the entry to the parasite into the cell. The mechanism of MASP-evoked calcium influx in the host cell membrane remains under investigation in our lab.

146 Functional analysis of the ATP synthase gamma subunit mutation that allows kDNA deletion in Trypanosoma brucei

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Energy metabolism in procyclic insect form (PF) and long slender bloodstream form (LS) Trypanosoma brucei is fundamentally different; this is reflected by a switch in the directionality of the mitochondrial F 1 F o -ATPase. In PF, the enzyme generates ATP via oxidative phosphorylation. In LS, ATP generated via glycolysis is used to drive proton pumping to maintain the mitochondrial membrane potential. Both functions are essential for the parasite. ATPase F o subunit 6 is critical for proton translocation in either direction and is encoded within the parasite's mitochondrial DNA (kinetoplast, kDNA), but naturally occurring dyskinetoplastic subspecies, such as T. evansi , are able to exist without kDNA. We have shown that a mutated F 1 ? subunit is sufficient to allow LS viability in the absence of kDNA. We currently investigate two questions: (i) What is the molecular mechanism of compensation for kDNA loss and (ii) where exactly in the parasite's life cycle does the switch in ATPase directionality occur? Surprisingly, we found that even in the presence of kDNA (and therefore F o), the mutation results in oligomycin resistance, suggestive of F 1 / F o uncoupling. We have generated LS cells expressing affinity-tagged ATPase subunits in cells and mutant ? and we will present data on how ? mutation and kDNA loss, respectively, affect the structure and function of the T. brucei ATPase. We also generated pleomorphic T. brucei with and without kDNA by expressing mutant g in strain AnTat 1.1. Preliminary studies investigating the capacity of these strains to differentiate suggests that kDNA is not required for formation of transmissible stumpy forms, but possibly for their viability.

147 TbNUP-2, a second component of the trypanosome nucleoskeletal lamina

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Lamins are required for a diverse array of cellular functions, from chromatin organisation and transcriptional regulation to nuclear structure and physical support for the cytoskeleton. However, lamin homologs are restricted to animals and amoeba, and absent from all other taxa. NUP-1 is a 450kDa coiled coiled protein at the nuclear periphery in African trypanosomes with functions highly similar to lamins, encompassing nuclear structure, chromatin organisation and transcriptional regulation, suggesting that it is a component of the trypanosome nuclear lamina. Recently, using cryomilling, immunoaffinity isolation and ESI MS/MS we identified NUP-2, which is also a coiled-coil protein. Both NUP-1 and NUP-2 interact with the nuclear pore complex. NUP-2 locates, in a punctuate distribution, to the nuclear periphery throughout the cell cycle, in close proximity to both NUP-1 and the telomeric ends of the chromosomes. RNAi-mediated silencing of NUP-2 leads to rapid and severe defects in proliferation, gross nuclear structure, chromatin organisation and nuclear envelope architecture. Further, transcription at telomeric-proximal sites, including VSG expression sites, is altered in NUP-2 depleted cells, suggesting a role in transcriptional regulation. However, unlike NUP-1, NUP-2 depletion did not lead to an increase in VSG switching. We suggest that NUP-2 is a second component of the highly unusual trypanosomatid nuclear lamina.

148 Regulatory mechanisms 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 definitive promoter and transcriptional control Leishmania depends on post-transcriptional and/or post-translational control for gene regulation. Recently we reported that Leishmania mitogen activated protein kinase2 (MPK2) regulates AQP1 stability through phosphorylation at the post-translational level. However, the role of post-transcriptional components and protein degradation pathways (post-translational) for regulating this important drug transporter is completely unknown. AQP1 mRNA contains a long 3'UTR (~ 1.8 kb). The 3'UTRs are established components of post-transcriptional regulation. They influence the mRNA stability as well as translational efficiency. 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. The stability studies revealed that AQP1 mRNA is unstable. The role of different controlling elements in the 3' UTR for AQP1 regulation will be discussed. Two major pathways for intracellular protein degradation are the ubiquitin-proteasome pathway and lysosomal proteolysis. Ubiquitination is a process in which ubiquitin molecules are attached to the target proteins by a series of steps namely E1, E2 and E3 ubiquitin ligases. Ubiquitinated proteins are destined to be degraded by proteasome. Leishmania has an E3 ubiquitin ligase called Anaphase Promoting complex/Cyclosome (APC/C) homologue in its genome. APC/C regulates stability of cell cycle regulators that are required for the progression of cell cycle. APC/C recognizes its substrates by RXXL motifs. We found three RXXL motifs in the protein sequence of AQP1. Two of them are conserved across all Leishmania species and are present at the cytosolic N-terminal loop. Role of these RXXL motifs in regulation of AQP1 will be also discussed.

149 Localization and Function of Palmitoyl Acyltransferase 7 in Trypanosoma brucei

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As is true in all eukaryotes, posttranslational modifications affect protein localization and function in Trypanosoma brucei . Palmitoylation, the addition of a 16-carbon fatty acid to a cysteine, is an essential modification in T. brucei . There are 124 palmitoyl proteins in T. brucei procyclic cells, which are involved in a number of important cellular roles including signaling and vesicle trafficking. Palmitoylation is carried out by the enzyme palmitoyl acyltransferases (PAT), of which T. brucei has twelve. Examination of individual TbPAT RNAi lines revealed that TbPAT7 is responsible for the palmitoylation of the flagellar calflagins. Inhibition of calflagin palmitoylation, either by mutation of the cysteine palmitoylation site or by TbPAT7 inhibition, causes calflagin to mislocalize to the pellicular membrane. PAT7 is found in the flagellar pocket of procyclic cells, which suggests a cellular trafficking pathway for the myristoyl-calflagins through the flagellar pocket and on to the flagellar membrane after palmitoylation. We hypothesized that additional substrates of TbPAT7 might also be flagellar. Therefore, we compared the palmitoyl proteomes of wildtype and TbPAT7 RNAi cells. A number of putative TbPAT7 substrates were identified, including the three calflagins, PI-PLC, and a putative t-SNARE protein, among others. Although global inhibition of palmitoylation kills trypanosomes, none of the individual TbPATs is essential for procyclic growth in vitro . We sought to determine the effect of PAT depletion in a mouse model of sleeping sickness. Mice infected with TbPAT7 RNAi line and treated with doxycycline were protected from sleeping sickness and death, with the parasites undergoing normal antigenic variation.

150 The rich repertoire of Leishmania major small nucleolar RNA: implication on their function in rRNA modification and processing.

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In eukaryotes, the two major base modifications are 2'-O-methylations (Nm) and pseudouridylation guided by C/D and H/ACA snoRNAs, respectively. Each RNP is bound by distinct subset of proteins and the modification is mediated by enzymes bound to the RNP; Fibrillarin (NOP1) for Nm and pseudouridine synthase (CBF5) for H/ACA RNA. We have previously reported on 23 clusters encoding for 62 C/D and 37 H/ACA RNA in L. major (Liang et al., Euk. Cell 2007). To complete the snoRNA repertoire as well as decipher the role of snoRNA in processes other than rRNA and snRNA modifications, we selected snoRNAs by affinity-purification of RNA associated with SNU13 (bound to C/D) and NHP2 (H/ACA). The selected RNAs were used to prepare small RNA libraries that were subjected to RNA–seq. 82 C/D and 86 H/ACA snoRNAs were identified. 27 of the H/ACA RNA, including a long H/ACA RNA (300 nt) are Leishmania-specific and for 15 molecules targets were not possible to predict bioinformatically. The study identified highly abundant snoRNAs that have a T. brucei homologue, and are implicated to direct trypanosome-specific rRNA processing. In contrast, we identified several L. major snoRNAs that share sequence similarity to a T. brucei snoRNA but are implicated to have different functions. Furthermore, we identified changes in the secondary structure between T. brucei and L. major rRNA that are compensated by snoRNA that direct modifications on these altered domains. Together, this study sheds light on the evolution of these important non-coding RNAs, revealing a much richer repertoire than many other unicellular eukaryotes, possibly because of these direct modifications that stabilize the ribosomes while cycling between the two hosts.

151 Leishmania infantum chagasi Ecto-Nucleoside Triphosphate Diphosphohydrolase: heterologus expression, biochemical characterization and influence on macrophage infection

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Leishmania infantum chagasi (synonym of Leishmania infantum) is the main pathogenic agent of Visceral Leishmaniasis in the New World. This protozoa parasite is unable to synthesize purine rings and is dependent of purine salvage pathways that use E cto-Nucleoside T riphosphate d iphosphohydrolases (E-NTPDases) to breakdown extracellular nucleotides. Recently E-NTPDases were pointed as virulence factors in many pathogenic agents by their ability to modulate hosts purinergic and pirimidinergic signaling that controls many biological process such as the immune system. In this work we showed for the first time the presence of Ecto-NTPDases in the surface of L. infatum chagasi . Furthermore we isolated, cloned and heterologous expressed in bacterial system a bioactive form of L. infantum chagasi E-NTPDase. The recombinant enzyme was named as NTPDase-2 and was purified and biochemically characterized. The enzyme was capable to use GTP, ADP and UDP as substrates at similar levels and GDP at lower level. The nucleotidase activity was magnesium dependent. The pH dependence of UDPase activity showed higher activities between pH 7 and 8. NTPDase-2 was partially inhibited by classical E-NTPDase inhibitors. The block of NTPDase-2 using specific antibodies and competition assay with recombinant NTPDase-2 leaded to lower levels of in vitro macrophage adhesion and infection. This work shows the L. infantum chagasi NTPDase-2 as a genuine apyrase from E-NTPDase/CD39 family and evidences a role of this ecto-enzyme in the parasite infection pointing it as new good target to rational drug design to treat Visceral Leishmaniasis. Supported by: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Pesquisa (CNPq), Instituto Nacional de Biotecnologia Estrutural e Química Medicinal em Doenças Infecciosas (INBEQMeDI) and Universidade Federal deViçosa.

152 Functional contribution of Pds5 to sister chromatid cohesion in T. brucei

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The ring-shaped cohesin complex entraps the sister chromatids to ensure correct chromosome segregation. The ability to form such linkage is tightly regulated during cell cycle and depends on loading and establishment factors that are associated with the cohesins. One of these factors is Pds5 (Precocious dissociation of sisters) whose sequence is well conserved during evolution and has apparently opposite roles in cohesin function depending on the model system. In this study, we identified the trypanosome orthologue of Pds5 and functionally characterized it with respect to cell cycle and chromatid cohesion. We first developed an antiserum against the C-terminal domain of TbPds5 that allowed us to analyze the subcellular localization and the expression pattern. TbPds5 was located in the nucleoplasm and expressed throughout late G1 to G2 phases. Co-immunoprecipitation assays performed using antibodies against TbScc1 and TbSmc3, two major subunits of the cohesin complex, demonstrated the interaction of TbPds5 with these core proteins. Partial depletion resulted in a growth defect with a significant S/G2 cell arrest, suggesting that TbPds5 function is essential. To understand TbPds5 function on chromatid cohesion, TbPds5 depletion was induced in several cell lines containing GFP-tagged loci. After 48 hours of Pds5 depletion, separation of sister chromatids was significantly delayed, suggesting that TbPds5 functions to facilitate cohesin release in G2/pre-mitotic cells, as shown in Xenopus and mammalian cells but in contrast to yeast and Drosophila. Finally, pull-down and proximity ligation assays using monoclonal antibody anti-TbSUMO showed that TbPds5 is SUMOylated, suggesting a role for this post-translational modification in the mechanism of TbPds5 regulation.

153 Phosphatidylethanolamine synthesis in Trypanosoma brucei

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Phosphatidylethanolamine (PE) is a major phospholipid class in eukaryotes. It can be synthesized by: i) phosphatidylserine (PS) decarboxylation, ii) base exchange with PS, and iii) the CDP-ethanolamine (Kennedy) pathway. In T. brucei , disruption of the Kennedy pathway leads to growth arrest of trypanosomes, demonstrating that PE formation by the Kennedy pathway is essential for parasite survival. In contrast, little is known about the contributions of the other two pathways to PE formation in T. brucei . We now show that RNAi-mediated downregulation of PS decarboxylase (PSD) affects growth of T. brucei procyclic forms. We localized PSD in the mitochondrion and found that its depletion affected mitochondrial membrane potential. Together, these results demonstrate that PSD is essential for parasite viability. However, labeling experiments using [3 H]serine showed no effect on de novo [3 H]PE formation. [3 H]PE can also be generated via incorporation of [3 H]serine into sphingolipids, followed by degradation by sphingosine-1-phosphate lyase (SPL) to form [3 H]ethanolamine-phosphate and incorporation into [3 H]PE by the Kennedy pathway. We have localized SPL in the (outer) mitochondrial membrane and found that its down-regulation by RNAi had no effect on mitochondrial function and parasite growth. However, [3 H]serine labeling experiments showed that SPL-depleted cells have decreased amounts of [3 H]PE, demonstrating that ethanolamine-phosphate formation by sphingolipid breakdown contributes significantly to PE formation in T. brucei .

154 Characterization of a heteromeric potassium channel essential for the bloodstream form of T. brucei

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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. By this in silico approach we identified two genes with a putative potassium channel function and studied them in more detail. We showed that down-regulation of both of these genes in the bloodstream form of the parasite by RNAi leads to a severe growth-phenotype and therefore these proteins are essential in the bloodstream form. By expression in Xenopus oocytes we could demonstrate that substantial outward currents at a membrane potential of -40 mV are exclusively observed when the two proteins were combined. We therefore think the two proteins form heteromeric potassium channels. Furthermore we could show that these channels are blocked by sub-milimolar concentrations of Ba2+-ions. 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 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 in T. brucei cells. For the localization of the two proteins within the parasites we constructed cell lines that can express tagged versions of the proteins. They will be tested by immunofluorescence experiments.

155 Using chromatin-based strategies to identify transcription-associated elements in Leishmania major

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Protein-coding genes in Leishmania and other trypanosomatids are transcribed polycistronically by RNA polymerase II (RNAPII) from long head-to-tail arrays called polycistronic gene clusters (PGCs). Transcription of PGCs initiates primarily within divergent strand switch regions (SSRs), which lack known eukaryotic RNAPII promoter elements. Chromatin-based strategies which are amenable to analysis by high throughput sequencing have been used to identify novel active regulatory elements in a number of model systems. In an effort to identify and characterize elements involved in transcription of PGCs, we used formaldehyde-assisted isolation of regulatory elements (FAIRE) and micrococcal nuclease (MNAse) digestion of chromatin to reveal nucleosome depleted and nuclease hypersensitive loci, respectively. Preliminary FAIRE-quantitative PCR (qPCR) analysis suggests that chromatin in divergent SSRs is nucleosome-depleted relative to chromatin near the middle of a PGC, and high-throughput sequencing analysis is underway to characterize this pattern genome-wide. MNAse-seq analysis demonstrates that tRNA and rRNA genes are highly sensitive to nuclease digestion in actively transcribing Leishmania promastigotes. In some experiments, we observe clear nuclease hypersensitivity in divergent SSRs and regions associated with acetylated histone H3 (H3Ac), but in others this pattern is not evident. The basis of these differences is under investigation. To correct for aneuploidy in Leishmania and account for sequencing artifacts and nuclease biases, we have developed a novel bioinformatics pipeline for processing MNAse-seq data which allows for normalization to MNAse-treated naked DNA controls. This pipeline facilitates the unbiased detection of nuclease hypersensitive loci and correlation of these loci to genome annotations.

156 Characterization of the Late Endosome and ESCRT Machinery in *Trypanosoma brucei*

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The eukaryotic late endosome (LE) is an endocytic compartment marked by the small GTPase, Rab7, and through which both biosynthetic and endocytic cargo pass en route to the lysosome. In Trypanosoma brucei TbRab7 regulates lysosomal trafficking of endocytic cargo, but not of normal biosynthetic cargo or engineered default lysosomal reporters, suggesting an alternate route to the lysosome might exist. The multivesicular body (MVB) is defined as a Rab7+ LE that has multiple intralumenal vesicles. The MVB functions in targeting ubiquitinylated cell surface proteins to the lysosome for degradation. Trypanosomes lack a morphologically defined MVB structure, but do contain orthologues of the ESCRT machinery that mediates MVB formation. We have investigated the role of two such proteins in lysosomal trafficking in bloodstream form T. brucei. Both TbVps23, an early ESCRT I component, and TbVps4, the ATPase that disassembles terminal ESCRT complexes, co-localize prominently with the TbRab7+ LE. RNAi silencing of each rapidly blocks growth. TbVps4 silencing results in ~3-fold accumulation of TbVps23 at the LE, consistent with blocking terminal ESCRT disassembly. Trafficking of endocytic and biosynthetic cargo, but not default reporters, is also negatively affected. Others have reported that TbVps23 silencing blocks ubiquitin-dependent lysosomal degradation of invariant surface glycoproteins (ISG) (Traffic 2008, 9:1698; EC 2011, 10:916). However, we find that TbVps23 silencing does not affect ISG65 turnover at all, while TbVps4 silencing actually enhances lysosomal degradation. In the absence of ESCRT function internalized ISG65 may be diverted into the alternate default pathway to the lysosome. These results confirm that the ESCRT machinery is essential in T. brucei and plays an important role(s) in LE function that is distinct from TbRab7.

157 Dual core processing: MRB1 is an essential trypanosome RNA editing complex

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Mitochondrial RNA editing in trypanosomes entails the usage of guide RNAs (gRNAs) as templates for uridine insertion and deletion into mRNAs by the RNA Editing Core Complex (RECC, a.k.a. editosome). However, recent studies have identified a second multiprotein complex that is also essential for RNA editing, called the Mitochondrial RNA Binding Complex (MRB1). Studies of MRB1 complex architecture through yeast two-hybrid and in vivo pull down experiments reveal that it comprises multiple dynamically associating subcomplexes. Functional studies demonstrate roles for these subcomplexes in gRNA stability, mRNA stability, RNA editing initiation and RNA editing progression. The epicenter of MRB1 is an RNA-independent core complex that binds gRNA, and repression of core components leads to defects early in the editing process, suggesting an essential role in RNA delivery to or positioning within the editosome. The MRB1 core complex has RNA-enhanced and RNA-dependent interactions with other subcomplexes including TbRGG2 subcomplex(es). The TbRGG2 subcomplex(es) modulate RNA editing initiation and 3' to 5' progression. RNA sequencing and in vitro biochemical studies are consistent with TbRGG2 complexe(es) playing a role in gRNA utilization, and in vivo complementation studies suggest that the RNA annealing activity of TbRGG2 is important in this process. A number of putative RNA binding proteins associate with the core complex and may act as editing specificity factors and/or connect the MRB1 complex to RNA regulatory steps beyond editing, such as processing and translation of RNA. Finally, an ARM/HEAT repeat protein that has a strong association with the core complex and TbRGG2 subcomplex(es) may act to coordinate protein-protein interactions within MRB1 and between MRB1 and other RNA processing machineries.

158 Trypanosoma brucei TIN2 inhibits VSG switching by maintaining the subtelomere integrity

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Trypanosoma brucei is a protozoan parasite and the causative agent of human African trypanosomiasis. T. brucei evades the host immune responses by regularly switching its surface antigen – Variant Surface Glycoprotein (VSG), which is exclusively expressed in a monoallelic fashion from VSG expression sites (ESs) located adjacent to the telomere. Telomeres are specialized nucleoprotein complexes at the ends of linear chromosomes and are essential for chromosome stability and genome integrity. Telomere functions are particularly important for antigenic variation in T. brucei. We have identified TbTIN2 as an intrinsic component of the T. brucei telomere complex. Using a cell line carrying both positive and negative selective markers in the active ES, we found that a temporary depletion of TbTIN2 led to a significant increase in the VSG switching frequency. Most of the VSG switchers in TbTIN2-depleted cells appear to arise through gene conversion events encompassing the entire active ES or loss of the active ES coupled with in-situ switching. Using ligation mediated-PCR, we observed many more DNA double strand breaks (DSBs) in the subtelomeric region upon depletion of TbTIN2. This suggests that wild-type TbTIN2 has an essential role in maintaining the subtelomere integrity. In addition, depletion of TbTIN2 led to an increase in the association of RAD51 with the subtelomeric DNA. RAD51 homologs in other organisms are involved in the strand invasion step during homologous recombination. Therefore, our observation suggests that DNA damage repair mediated by TbRAD51 through homologous recombination led to subsequent increased VSG switching frequency.

159 A calpain-like protein regulates cell shape in Trypanosoma brucei

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The shape and form of kinetoplastids varies significantly between species as well as during the life cycle of an individual species. These different shapes have been categorised based on the position of the flagellum, basal body and the length of flagellar attachment. However, little is known about the processes which define and enable these radical rearrangements. We have identified a large calpain-like protein that contains numerous GM6 repeats (ClpGM6) that is involved in determining cell shape and form in T. brucei. ClpGM6 is a cytoskeletal protein that localises within the flagellum along the length of the flagellar attachment zone. Reduction of the ClpGM6 amount in procyclic cells by RNAi leads to a shortening of the flagellar attachment zone. This is accompanied by repositioning of kinetoplast, which is found anterior to the nucleus under these conditions. The cells have therefore changed their trypomastigote layout to an epimastigote-like one and indeed some cells have an almost promastigote appearance. Importantly there is no change in growth rate of these cells and they can be kept in an induced state for months without the loss of the epimastigote-like morphology. Based on these observations we conclude that the major changes in cell shape in the life cycle of kinetoplastid parasites are likely to be caused by the modulation of expression of a small number of genes, Also, we conclude that the different forms acquired during kinetoplastid evolution are unlikely to be the result of large scale changes in gene complement.

160 The dynamic nature of experimental chronic Chagas disease revealed by highly sensitive in vivo imaging

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Chronic Trypanosoma cruzi infections either remain asymptomatic or result in cardiac and/or digestive pathologies. During the chronic stage of Chagas disease parasites are thought to be sequestered in diverse tissues, but links between parasite tissue tropism and disease pathogenesis have been poorly defined. To address this we developed a real-time bioluminescence imaging infection model based on a transgenic T. cruzi cell line, constitutively expressing the ‘red-shifted’ firefly luciferase variant Ppy RE9. Luciferase expression levels were tightly correlated with parasite number and significant reduction of bioluminescence was observed after oral treatment of acute and chronic infections with benznidazole. The in vivo limit of detection was <1000 parasites per animal and therefore vastly more sensitive than peripheral blood parasitaemia counts. In a model of acute fulminating disease in an immunuocompromised host (SCID mouse) ex vivo imaging of tissues showed the highest parasite burden to be harboured by visceral fat depots. Parasite burdens in immunocompetent BALB/c mice peaked at 14 d.p.i. and could be visualised for >250 days. Chronic parasite foci were highly dynamic with a high degree of spatial variation and parasite burden intensity fluctuating over 2 logs of magnitude. Ex vivo imaging revealed that the large intestine was the primary site of chronic (153 dpi) parasite persistence with 5 to 12-fold higher burdens compared to the heart. Nevertheless, mice developed myocarditis and progressive heart fibrosis. These data imply that chagasic cardiac pathology may not result exclusively from local parasitism and the specific immune response directed against it.

161 The VSG 3'UTR and regulation of VSG expression

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T. brucei has a vast repertoire of Variant Surface Glycoprotein (VSG) genes, of which only one is expressed at a time from one of approximately 15 sub-telomeric VSG expression sites. VSG constitutes about 10% total cellular protein, however it is unclear how these high expression levels are achieved from a single VSG gene. There is little transcriptional control in T. brucei , and mRNA stability sequences function as a key point of control. We are currently investigating the regulatory role of the VSG 3'UTR on levels of expression of VSG. We have shown that T. brucei can express a second VSG (VSG117) from a location immediately downstream of the active VSG221 expression site promoter, leading to trypanosomes with a mixed VSG coat. Deletion of the telomeric VSG221 leads to VSG117 single-expressors, showing that adequate levels of VSG can be expressed from a non-telomeric copy. Alternatively, blocking synthesis of one VSG using RNAi, can make the trypanosome reliant on the other VSG. We are using this system to examine the role of the VSG UTR, and have inserted copies of VSG117 where the VSG 3'UTR is replaced with a tubulin UTR leading to significantly lower levels of RNA expression. In addition, a second VSG (VSG117) with either a VSG 3'UTR or a tubulin 3'UTR has been expressed from a variety of other PolI and PolII transcribed genomic loci (the tubulin array, the procyclin array and the rDNA array). We are currently determining the role of both genomic location and RNA stability sequences for optimal expression of VSG.

162 Interaction of Leishmania donovani PEX14 with glycosomal membrane

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Leishmania contain a unique glycosomal organelle that compartmentalizes a variety of vital metabolic pathways. G lycosomal proteins are synthesized in the cytosol and post-translationally imported into the glycosome via a C-terminal PTS1 or N-terminal PTS2 signal sequence that is bound by the receptor proteins PEX5 and PEX7, respectively, and bind to the glycosomal membrane associated protein PEX14. The Leishmania PEX14, is peripheral membrane protein that is anchored to the cytosolic face of the glycosomal membrane, and contain a variety of functional and structural domains that include binding sites for the receptor proteins PEX5 and PEX7, a leucine zipper that mediates PEX14-PEX14 interactions, and a hydrophobic region required for membrane association. Previous studies demonstrated that the hydrophobic region spanning residues 120-200 were essential for the binding of PEX14 to liposomes mimicking the phospholipid composition of the L. donovani glycosomal membrane. Moreover, using dye leakage assays it was shown that a fragment spanning this region, pex14(120-200), was capable of forming a pore in the liposome bilayer. To further investigate this protein-membrane interaction we generated a panel of mutants in which a tryptophan residue was inserted at various positions of the hydrophobic region. Intrinsic fluorescence studies performed using the quenching agents potassium iodide and 10-doxylnonadecane were carried out to examine the topology of this peptide in the lipid bilayer and to assess the depth to which the tryptophan inserted into the hydrophobic core of the membrane. Our studies support the notion that this hydrophobic region which favors adopting an amphipathic helical conformation is critical not only for anchoring PEX14 to the glycosome membrane, but also in the transport of protein cargo into the glycosome.

163 Correlating lifestyle and trafficking systems in kinetoplastids: The evolutionary history of Rab and SNARE proteins

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Life-cycle progression and pathogenic mechanisms in kinetoplastid parasites depend substantially on their intracellular trafficking systems. Bloodstream form Trypanosoma brucei uses antigenic variation and an extremely active endocytic apparatus for immune evasion. Leishmania and T. cruzi are predominantly intracellular, but can rapidly remodel their surfaces to display an array of variable antigens with roles in invasion and immune evasion. Genome-wide sequence data for a number of parasitic kinetoplastids are becoming available, along with data from the free-living relative Bodo saltans . We have exploited these resources to enquire how intracellular trafficking varies across this lineage. We report an inverse correlation between the presence of VSG and the AP-2 complex, suggestive of coordinated evolutionary processes. Members of Rab GTPase and SNARE protein families in twenty Euglenid and Heterolobosid genomes were identified using domain-based and sequence-based searches for these central trafficking protein families. This, together with phylogenetic reconstruction revealed the following evolutionary patterns: Overall the Rab and SNARE families we found to be stable across the Euglenids, with little evidence for large-scale expansions or contractions accompanying either parasitism, radical alterations in host, host defense systems, environment or transmission mechanisms. However, significant smaller scale changes were observed, and these will be discussed.

164 Environmentally regulated localization of Trypanosoma brucei hexokinase 2

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Trypanosoma brucei hexokinase 2 (TbHK2) bears a peroxisomal targeting sequence signal but localizes to both the glycosomes and the flagella in bloodstream form (BSF) parasites and to the glycosomes and proximal to the basal bodies in procyclic form (PF) parasites. The mechanism behind the extra-glycosomal localization of this enzyme, which can catalyze the first step in glycolysis, is unknown. Previously, we have found that the level of cellular HK activity is modulated based on environmental carbon source. Here, we report that TbHK2 expression and localization is altered in response to glucose. By immunofluorescence and sub-cellular fractionation, we have found that the distribution of TbHK2 is dynamic in a glucose-dependent fashion, with greater extra-glycosomal distribution when cells were grown in the presence of glucose. Further, we have evidence from proteomic studies that in PF parasites, TbHK2 becomes associated with the mitochondria in response to overexpression of T. brucei voltage-dependent anion channel (TbVDAC). Together, the environment-dependent localization and association with mitochondrial function suggests a role for TbHK2 as a central regulator of metabolism, connecting glycolysis to mitochondrial activity.

165 Investigation of cis-elements involved in regulation of gene expression in Leishmania

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In trypanosomatids, the regulation of the gene expression occurs mostly at the post-transcriptional level. Thus, the stability of the RNA and the rate of translation initiation are important levels of control of gene expression in these parasites. Cis-elements present in 3' and/or 5' untranslated regions (UTRs) of Leishmania transcripts likely play a central role in the control of mRNA stability and/or translation initiation by interacting with regulatory proteins. Comparative analysis of Leishmania genomes of distinct species revealed that highly conserved coding sequences (CDS) are accompanied by poorly conserved inter-CDS sequences. The identification of conserved sequence motifs in a divergent genomic landscape may lead to the discovery of new functional cis-elements. Therefore, to identify the elements involved in the control of gene expression, we conducted an in silico investigation to find conserved intercoding sequences (CICS) in the genomes of L. major, L. infantum, and L. braziliensis to search and classify the CICS (Vasconcelos et al, MBP, 2012). We selected five of the thousands of novel CICS identified to investigate their putative functional role using reverse genetics. To investigate whether these CICS are necessary and sufficient to control the transcript levels we inserted the CICS in the 3'UTR of a constitutively expressed gene (DHFR-TS) and removed it from its original locus in L. donovani. In both cases, neo was used as selectable marker and reporter gene; with both transcript and protein levels being evaluated throughout the life cycle of mutants. We also analyzed proteins that interacted with these CICS using a RNA pull-down assay with L. donovani nuclear extract and mass spectrometry. These studies revealed two different RNA binding proteins that interacted with both CICS.

166 Host fatty acid metabolism and growth of intracellular Trypanosoma cruzi amastigotes

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The intracellular amastigote stage of Trypanosoma cruzi is a critical target for vaccine and drug development for the prevention and treatment of human Chagas' disease. Despite their importance, little is known regarding cellular and metabolic pathways that fuel intracellular growth and survival of T. cruzi amastigotes in mammalian host cells. A recent genome-wide RNA interference screen conducted in HeLa cells identified interconnected metabolic networks centered around host energy production, nucleotide metabolism, pteridine biosynthesis, and fatty acid oxidation as key processes that fuel intracellular T. cruzi growth. Here, we present functional follow-up studies aimed at addressing the role of host fatty acid metabolism in supporting T. cruzi amastigote replication. Our previous results implicate both peroxisomal and mitochondrial fatty acid oxidation pathways in the host as modulators of intracellular amastigote growth. Whether T. cruzi amastigotes exploit fatty acid intermediates generated via host oxidative pathways or whether they benefit indirectly from the production of energy and reductive intermediates formed in these catabolic processes remains to be determined. To address the first possibility, we examine the ability of exogenous fatty acids (C12-C24) to rescue T. cruzi amastigote growth restriction in mammalian cells with compromised capacity for either peroxisomal or mitochondrial fatty acid oxidation eg. following silencing of host pyruvate dehydrogenase kinase 4 (PDK4) and in primary human cells deficient for peroxisomal biogenesis (Zellweger's) or mitochondrial long chain fatty acid oxidation (ACADVL-deficient). The ability to detect trafficking of BODIPY-FL-C16 and C12 to intracellular amastigotes in infected fibroblasts indicates the feasibility of this approach. These studies provide the foundation for metabolic flux studies to determine fates of long-chain fatty acids and their role in supporting T. cruzi infection.

167 Novel Therapeutics for Human and Animal African Trypanosomiasis

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African trypanosomes are the causative agent of sleeping sickness in humans and Nagana, a wasting disease, in African and South American cattle. Recently we have developed and characterized several small hydrophobic peptides (SHP) that specifically kill bloodstream developmental form (BSF) African trypanosomes by increasing the rigidity of the plasma membrane. Human pathogenic Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense and major animal pathogens such as Trypanosoma brucei brucei , Trypanosoma vivax and Trypanosoma congolense , are susceptible to killing by SHP. Other developmental forms and human cell lines, including erythrocytes, are refractory to killing by SHP at concentrations orders of magnitude higher than necessary to kill BSF African trypanosomes. Specificity of SHP for BSF trypanosomes is mediated by a high degree of fluidity in the plasma membrane. Our data indicates that lipid bilayer fluidity is an essential component of BSF physiology that may be targeted by drugs. Here we describe studies utilizing nanoparticle and liposome delivery platforms to develop new trypanocidal formulations based upon these novel antimicrobial peptides.

168 Implication of Leishmania MAPKs in miltefosine resistance

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The protozoan parasite Leishmania is the causative agent of leishmaniasis, a disease that affects around 12 million people worldwide, with 1.5–2 million new cases each year. Miltefosine (MIL), the only leishmanicidal drug orally active on visceral (VL) and cutaneous leishmaniasis (CL), is currently administered as an alternative to antimonials in areas where resistance to these drugs is rampant. Although no MIL-resistant clinical isolates have been yet officially reported, they can be anticipated given the long half-life of the drug and extended treatment courses. The overexpression of an active Mitogen Activated Protein Kinase 7 (MPK7) confers resistant to miltefosine in a specific fashion. Therefore, MAPKs and their downstream targets may play an important role in drug resistance in Leishmania. We characterized defects in membrane potential and miltefosine uptake/efflux using fluorescent analogs of MIL. Notably, Real Time Quantitative RT-PCR studies revealed similar expression levels of the Leishmania miltefosine transporter LdMT and its subunit, LdRos3, in both resistant and control parasites. Additionally, we compared susceptible wild type parasites with MIL resistant lines established in vitro in our laboratory by continuous step-wise drug pressure and MPK7 overexpressing parasites. We characterized the resistant cell lines in terms of apoptosis and EC50 values. Two different apoptotic markers were used: membrane permeability (YO-PRO) and PE exposure (Annexin V). Finally, we carried out comparative phosphoproteomics of sensitive and resistant lines with the aim to identify molecules implicated in the resistant phenotype. This work was supported by projects PI 12-02706 and RD12/0018/0007 (ISCIII) to L.R.

169 The role of KBP17, a novel Trypanosoma brucei kDNA-binding protein, in kinetoplast DNA replication.

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The mitochondrial genome in T. brucei, called kinetoplast DNA (kDNA), is a complex network of catenated minicircles and maxicircles, compacted into a disk-shaped structure adjacent to the flagellar basal body. During replication, minicircles are released from the network, replicate, segregate to two antipodal sites flanking the kinetoplast disk, and then are reattached to the network. In order to better understand this elaborate pathway, we have used a proteomic approach to search for new kDNA replication proteins. Following DNA-affinity purification and mass spectrometry analysis of mitochondrial proteins, we identified a small, basic 17kDa protein, distantly related to metazoan H1 histones. Based on immunolocalization studies showing that this protein is located on the kDNA, we have named it K inetoplast B inding P rotein 17 (TbKBP17). Although TbKBP17 colocalizes with kDNA throughout the cell cycle, it preferentially associates with minicircles—either newly replicated molecules located at the antipodal sites or those reattaching to the network. Knockdown of TbKBP17 by RNAi causes defects in the kDNA condensation and leads to the production of ancillary kDNA networks in different regions of the mitochondrion. TbKPB17 overexpression leads to the shrinkage or loss of kDNA, and an arrest in cell growth. Remarkably, overproduction causes minicircles to reattach throughout the kDNA disk instead of at the poles, leading to altered kinetoplast structure. Recombinant KBP17 binds double-stranded circular DNA, condenses purified kDNA, and stimulates topoisomerase II catenation activity in vitro . We speculate that TbKBP17 is a DNA-condensing factor for the topoisomerase II-mediated reattachment of minicircles at the antipodal sites.

170 Leishmania-macrophage interactions: novel mechanisms of antioxidant/antinitrosative defense.

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Professional phagocytes generate reactive oxygen (ROS) and nitrogen species (RNS) to kill invading microorganisms. Many intracellular pathogens have developed evasive strategies against the microbicidal nature of ROS/RNS, including the expression of detoxifying enzymes, scavengers and/or repair systems, promoting their own survival within the harsh intracellular environment. Intriguingly, Leishmania parasites lack many of the classical detoxifying enzymes present in other intracellular pathogens, such as catalase or selenocysteine-containing glutathione peroxidase. Therefore, they primarily rely on trypanothione and trypanothione-dependent peroxidases to defend against both oxidative and nitrosative stress. Although nitric oxide (NO) can promote the clearance of intracellular parasites, some Leishmania species are relatively resistant to NO-mediated antimicrobial activity, but the underlying resistance mechanisms remain unknown. Here, we show that L. amazonensis, a species that causes severe infection in humans and non-healing skin lesions in mice, responds to nitrosative stress by secreting a discrete subset of proteins, including a unique tryparedoxin peroxidase (TXNPx) known to detoxify RNS. Interestingly, TXNPx expression is limited to the intracellular environment of macrophages, but can be induced by parasite exposure to authentic RNS in vitro. The TXNPx-containing supernatant from NO-treated parasites exhibits increased antioxidant capacity compared to that of untreated parasites. This conditioned parasite supernatant limits NO production by cytokine-activated macrophages while increasing intracellular parasite survival. Collectively, these data indicate that L. amazonensis parasites can actively respond to RNS by secreting proteins that antagonize host cell antimicrobial activity, which allows their intracellular survival and persistent infection in the host.

171 Cell surface proteomes from insect-form and mammalian bloodstream-form Trypanosoma brucei

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African trypanosomes (Trypanosoma brucei and related subspecies) are devastating human and animal pathogens that cause sleeping sickness in humans and limit economic development throughout much of sub-Saharan Africa. Trypanosomes are transmitted between a range of mammalian hosts by the tsetse fly and the parasites must sense and respond to diverse extracellular signals for survival, transmission, and pathogenesis. Recent efforts from multiple groups have elucidated how the T. brucei transcriptome and proteome change as parasites differentiate and adapt to their varied external environments and life cycle stages. However, only a few cell surface proteins have been characterized and the full repertoire of surface protein diversity and functionality remains unknown. As the interface with the host environment, cell surface proteins are critical for the parasitic life-style of T. brucei. Moreover, they also impact the success or failure of therapeutic interventions for sleeping sickness. We have conducted proteomic analyses of the T. brucei cell surface from insect-form and mammalian bloodstream-form parasites. In addition to identifying many of the known surface proteins, these analyses uncovered proteins of unknown function that are life cycle stage-specific and may provide host-specific adaptations. This work complements existing expression studies by providing an assessment of surface exposure, as well as accessibility to small molecules. Further characterization of individual life cycle stage-specific surface proteins will improve our understanding of host-parasite interactions and may open new avenues for therapeutic intervention in sleeping sickness.

172 Fucosylation in Trypanosoma brucei

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In the last decade the discovery of an essential GDP-fucose de novo pathway in Trypanosoma brucei inevitably raised the question for downstream enzymes utilizing GDP-fucose1. This prompted us to further investigate putative T. brucei fucosyltransferases and fucose containing glycoconjugates (fucotopes). In this cooperative study we identified a single fucosyltransferase (FT) in the T. brucei genome, which was recombinantly expressed and analysed, demonstrating the presence of a GDP-Fuc:β-D-Gal α1,2-fucosyltransferase. Furthermore, we generated an FT conditional gene deletion mutant in procyclics and performed an RNAi knock-down in blood stream form parasites which substantiate the essential relevance of FT expression for cell growth in culture. On the other hand, T. brucei fucotopes still remain enigmatic with the exception of a recently discovered high molecular weight glycoconjugate in the procyclic stage2. Unfortunately, so far it was impossible to identify the fucose acceptor substructures of this complex or to characterise other fucotopes. Due to the absence of a GDP-fucose salvage pathway in T. brucei, biosynthetic radiolabelling with [3H]-fucose is not feasible. However, since this strategy poses the most powerful approach to tackle fucosylated glycans, we implemented an artificial GDP-fucose salvage pathway. Therefore the recently characterised bifunctional arabinose/fucosekinase/pyrophosphorylase (AFKP80), catalysing the biosynthesis of GDP-arabinose and GDP-fucose in L. major was recombinantly expressed in T. brucei bloodstream form parasites. This strategy allowed us to radiolabel fucotopes as shown by an autoradiograph of cell lysates, indicating fucose containing high molecular weight glycoconjugates in the blood stream form and other still unknown fucose containing glycoproteins. Additionally, we learned thereby that T.brucei is able to transport fucose across its plasma membrane. [1] Turnock DC, Izquierdo L, & Ferguson MA (2007); J. Biol. Chem., 282, 28853-28863. [2] Guther ML, Beattie K, Lamont DJ, James J, Prescott AR, & Ferguson MA (2009); Eukaryot. Cell, 8, 1407-1417.

173 Use of the HaloTag to modulate protein level in Trypanosoma cruzi and Trypanosoma brucei.

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A wide variety of drug targets have been assessed in Trypanosoma brucei fuelling successful drug discovery campaigns in both academic and industrial settings. By contrast there is a scarcity of validated drug targets in Trypanosoma cruzi in part due to a paucity of genetic tools for assessing essentiality. RNAi is not possible in T. cruzi and no conditional expression system is in wide use. We have used the HaloTag degradation system previously used in higher organisms [1] to target luciferase for destruction in T. cruzi. This system uses small ‘tagging’ molecules which irreversibly bind to a HaloTag fusion protein. The tagged protein displays a hydrophobic moiety, which causes it to be degraded via the proteasome depleting the target much more rapidly than expression-targeting approaches. Using a cytotoxicity assay optimised for epimastigotes together with a high-content screen for intracellular amastigotes, we have synthesised and identified suitable tagging compounds and established optimum conditions to induce degradation of luciferase in T. cruzi . We have also assessed the system in T. brucei. Whilst generation of conditional null mutants is relatively straightforward in these parasites, the HaloTag system can be used for target assessment in vivo. Our approach could provide a more realistic surrogate for small-molecule mediated inhibition of a target, where complete inhibition is an inadvisable goal to set during target assessment. A similar strategy for Leishmania spp is under development. [1] Neklesa TK, Tae HS, Schneekloth AR, et al. Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. Nat Chem Biol 2011;7:538-43.

174 Trypomastigotes and amastigotes of Trypanosoma cruzi induce apoptosis and STAT3 activation in cardiomyocytes in vitro

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The haemoflagellate Trypanosoma cruzi is the causative agent of Chagas' disease that occurs in approximately 8 million people in Latin America. Patients infected with T. cruzi frequently suffer of cardiomegaly and may die of myocardial failure. Here we show that T. cruzi trypomastigotes (extracellular form) increased in vitro apoptosis of rat cardiomyocytes. Additionally, we demonstrated that amastigotes (intracellular form), for which a method for purification was established, were also able to induce cardiomyocyte apoptosis. Increase of apoptosis was associated with up-regulation of the apoptotic gene bax by trypomastigotes, while expression of the anti-apoptotic gene bcl-2 was down-regulated by amastigotes. The transcription factor STAT3 but not STAT1 was activated in cardiomyocytes by trypomastigotes. In addition, tlr7 gene expression was up-regulated in cardiomyocytes incubated with trypomastigotes, suggesting that this Toll-like receptor is involved in the intracellular recognition after host cell invasion by T. cruzi. Glycosylphosphatidylinositols purified from trypomastigotes did not induce cardiomyocyte apoptosis and STAT activation but down-regulated tlr7 gene expression. In conclusion, cardiomyopathy observed in Chagas’ disease might be in part due to apoptosis of cardiomyocytes induced directly by the parasite.

175 Stage-specific Differences in the Regulation of T. brucei Acetyl-CoA Carboxylase by Environmental Lipids

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Trypanosoma brucei readily acquires fatty acids from its hosts. When host supply is limited, T.brucei synthesizes its own fatty acids. Environmental regulation of FAS would allow preferential use of host fatty acids, and turn on FAS only when host supply is insufficient. A key FAS substrate is malonyl-CoA, which is synthesized by Acetyl-CoA Carboxylase (ACC). We hypothesize that TbACC is regulated in response to environmental lipids, as it is a likely regulatory control point for FAS. When T. brucei procyclic forms (PF) were grown in low lipid media, we observed a 2-fold increase in TbACC protein and activity. Addition of exogenous fatty acid significantly reduced TbACC activity. qPCR analysis showed no significant change in TbACC mRNA levels, suggesting post-translational regulation. Using [32P]phospholabeling and phosphoprotein staining, we showed that TbACC-myc cells demonstrated a 300-500% increase in TbACC-myc phosphorylation in high lipid media and an 80% reduction in low lipid media. Subsequent phosphatase treatment reduced phosphorylation and increased TbACC activity. Similar studies performed with bloodstream forms (BFs) revealed no change in TbACC mRNA level, protein level, or activity in response to environmental lipids. Interestingly, the green tea catechin, epigallocatechin gallate (EGCG), a known inducer of ACC phosphorylation, induced TbACC phosphorylation and decreased activity in both PFs and BFs (Vigueira et al., 2012). Our current model is that TbACC is differentially regulated by phosphorylation in PFs and BFs: In PFs, TbACC is dynamically regulated in response to the environmental lipid supply; in BFs, it appears TbACC is not regulated by environmental lipids, existing primarily in the dephosphorylated, active state.

176 An aminoacyl-tRNA synthetase complex is required for efficient tRNA-aminoacylation in African trypanosomes

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Aminoacyl-tRNA synthetase (aaRSs) enzymes are responsible for charging specific tRNAs with their cognate amino acids and are essential for accurate translation of the genetic code. In eukaryotes, aaRSs are organized in a multi-protein complex called the m ulti- a minoacyl-t R NA s ynthetase (MARS) complex, which is involved in tRNA channeling to ribosomes, translational control and cell signaling. Trypanosoma brucei have 25 genes annotated as aaRSs. Most of the enzymes that they encode localize to the cytoplasm and are essential for parasite growth as determined using RNAi analysis. We found through the use of Tandem-Affinity Purification and mass spectrometry that T. brucei bloodstream and procyclic forms have a MARS complex composed of nine aaRSs and two related proteins. Glycerol gradient analysis showed that T. brucei MARS complex sediments between 10 and 20S. Monomeric and complex-associated methionyl-tRNA synthetase (MetRS) were purified by biochemical methods and analyzed enzymatically. Steady-state kinetic analysis indicated a high efficiency in tRNA Met aminoacylation for complex-associated MetRS as a result of a decreased K m for tRNA Met . In addition, the high efficiency in tRNA Met aminoacylation by the MARS complex is facilitated by a tRNA-binding protein (TRBP) that associates with the complex. Aminoacylation assays with MetRS in the presence of recombinant TRBP showed a dose-dependent increase in tRNA Met aminoacylation, suggesting that TRBP may act as a co-factor that binds and facilitates tRNAs aminoacylation by the complex. Conditional repression of the trbp gene in bloodstream form resulted in slow parasite growth, likely as a result of inefficient aminoacylation. Overall, T. brucei aaRSs are essential enzymes which are associated in a cytoplasmic MARS complex. This complex is required for efficient tRNA aminoacylation and contributes to parasite fitness.

177 Genomic analysis of sequence-dependent DNA curvature in Leishmania

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Genomic analysis of sequence-dependent DNA curvature in Leishmania Pablo Smircich 1,2 , Diego Forteza 1 , Najib M. El-Sayed 3 and Beatriz Garat 1 1 Laboratorio de Interacciones Moleculares, Facultad de Ciencias, 11400 Montevideo, Uruguay; 2 Departamento de Genética, Facultad de Medicina, 11800 Montevideo, Uruguay; 3 Department of Cell Biology and Molecular Genetics and Center for Bioinformatics and Computational Biology, University of Maryland College Park, MD 20742, USA Leishmania major is a flagellated protozoan parasite of medical importance. Like other members of the Trypanosomatidae family, it possesses unique mechanisms of gene expression such as constitutive polycistronic transcription of directional gene clusters, gene amplification, mRNA trans-splicing, and extensive editing of mitochondrial transcripts. The molecular signals underlying most of these processes remain under investigation. In order to investigate the role of DNA secondary structure signals in gene expression, we carried out a genome-wide in silico analysis of the intrinsic DNA curvature. L. major genome revealed a lower frequency of high intrinsic curvature regions as well as inter- and intra- chromosomal distribution heterogeneity, when compared to prokaryotic and eukaryotic organisms. Using a novel method aimed at detecting region-integrated intrinsic curvature (RIIC), high DNA curvature was found to be associated with regions implicated in transcription initiation. Those include divergent strand-switch regions between directional gene clusters and regions linked to markers of active transcription initiation such as acetylated H3 histone, TRF4 and SNAP50.These findings suggest a role for DNA curvature in transcription initiation in Leishmania supporting the relevance of DNA secondary structures signals.

178 Targeting Protein Kinases in Trypanosoma brucei

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We used an experimental genetic approach to assess the essentiality of potential drug targets in bloodstream form (BF) Trypanosoma brucei . We did so by making null mutants, by conditional knockdown of gene expression by RNAi, and by knockdown of transcription in conditional null mutants, i.e. null for endogenous alleles with a tet-regulatable ectopic allele. Overall we analyzed 142 genes of the most promising potential drug targets according to the tdrtargets.org database. Quantification of RNAi knockdown for 60 genes by quantitative real-time PCR revealed a wide range of knockdown with a median of 60% knockdown. The uncertainty of sufficient reduction of gene expression by RNAi led us to develop an alternative efficient method to generate null and/or conditional null mutants which eliminates most construct cloning. We used this approach to examine 30 protein kinases and identified six of those that are essential in BFs. Most essential kinases identified are homologs of CDC2-related kinases. Several kinases that we tested that had a loss of fitness in high-throughput RNAi studies (Alsford, et al. , Genome Res 21:6,915)are clearly not essential since we produced viable null mutants for these. Conversely, we identified kinases that are essential in BFs although RNAi did not have a growth defect. To date 142 genes across many classes of enzymes have been analyzed by our labs and we have identified 42 that are essential in BFs. A database of these results that is approaching completion will be made publicly available. Overall, these results show the utility of the null and/or conditional null approach to identify essential kinases which are promising therapeutic targets in T. brucei and related parasites.

179 Computational analyses of alternative trans-splicing in Trypanosoma brucei

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The genes in Trypanosoma brucei are transcripted as polycistronic pre-mRNA and joined with a spliced leader RNA through a trans-splicing process. Recent studies using high throughput sequencing of T. brucei transcriptom revealed that most genes are alternatively trans-spliced to generate different isofroms with distinct 5' end. The trans-splicing of many genes are tightly regulated in different life cycle stages of T. brucei, however little is known about how T. brucei select alternative 3' splice site to generate different isoforms. We seek to infer the regulation of trans-splicing using computational analyses of the sequence features in the published RNA-seq data. We found that nearly half of alternative splice sites are within short distance to each other (separated by less than 10 nt), suggesting that they may be resulted from inaccurate recognition of 3' splice site and have little consequence in protein translation. In particular, many of these alternative sites have consensus of AGAG or NAGNAG, probably reflecting the flexibility of 3' splice site selection by splicing machinery. Consistent with previous finding, we found that the upstream alternative sites are preferably used, and this preference cannot be fully explained by the splice site strength. We derived the maximum entropy model of alternative splice sites and used it to score pseudo- splice site along the transcripts, and compared the branch site sequences in alternative splice sites. In addition, we used a series of statistical analyses to identify splicing regulatory cis-elements for trans-splicing, and examined the possibility of co-regulation between alternative polyadenylation and trans-splicing. Taking together, these analyses shed light on how the alternative trans-splicing is regulated in T. brucei.

180 Development and characterization of inhibitors against essential trypanosome hexokinases

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Hexokinase (HK) is the first enzyme in the glycolytic pathway and is essential to the survival of Trypanosoma brucei. A high throughput screen for TbHK1 inhibitors identified several potential scaffolds for further consideration. Several inhibitors developed from the first scaffold, a benzamidobenzoic acid, have demonstrated potent inhibition of rTbHK1 in vitro, leading to the development of a probe inhibitor (ML205, SID 99437306, IC 50 = 976 nM). However, this compound lacked activity against parasites. Modifications to ML205 have been explored, yielding compounds with improved antiparasitic activity against blood stream form parasites (LD50 = 1.88 u M). Several isobenzothiazolinones were also identified in the initial HTS. However, advances using these compounds have been limited, as a structure activity relationship for this scaffold has not been established. However, we have used this class, and a derivative of this class called ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one), to investigate enzyme mechanism. While ebselen can inhibit enzymes by covalent modification of Cys residues, site directed mutagenesis of TbHK1 Cys residues, along with biochemical characterization of these variants, suggests the compound interacts with TbHK1 independently of the Cys residues. Additionally, alteration of the Cys residues has only modest impact on overall protein structure, as scored by oligomerization and electron microscopy. The mode of action of ebselen, as well as isobenzothiazolinones, is distinct from that found with other HKs, including a HK from Plasmodium falciparum.

181 Optimizing RNAi activity for study of the Leishmania flagellum

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RNA interference (RNAi) is an established method for functional genetic analysis, and we recently showed that Leishmania (Viannia) species have an active RNAi pathway able to down-regulate reporter and endogenous parasite genes (Lye et al., PLoS Pathogens 2010). We have established conditions for optimizing RNAi activity and developed vectors for the rapid generation of stem-loop (StL) constructs using Gateway (Invitrogen®) technology. We are now using these tools to probe the role of two conserved flagellar pathways, intraflagellar transport (IFT) and Bardet-Biedl syndrome complex (BBSome). We were unable to generate StL transfectants for four key IFT genes, while controls including PFR1-StL were successful. We transfected the same StL constructs into an Argonaute null mutant lacking a functional RNAi pathway, and these transfectants were viable. These data suggest that the IFT pathway is essential in L. braziliensis promastigotes. By successively reducing the length of the ‘stem’ from 940 to 131 nt, we obtained viable StL constructs for LbrIFT140 which showed phenotypes similar to Trypanosoma brucei IFT knock downs (Adhiambo et al., J. Cell Sci. 2010). This RNAi ‘hypomorphic’ strategy enables studies of IFT in viable promastigotes in the future. In contrast to IFT gene knock down, transfectants of StL constructs targeting three respective BBSome genes (BBS1, ARL6, and BBS4) readily yielded transfectants with WT morphology and growth. The effects on mRNA levels are being assessed for these BBSome transfectants. This result is consistent with recent reports where only modest effects on flagellar length were observed upon knock down of ARL6 in T. brucei (Price et al., BBA 2012).

182 Does Hemoglobin-Induced Peroxidation of Trypanosome Lytic Factor-1 Lead to Trypanosome Lytic Factor-2 Biogenesis?

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Trypanosome Lytic Factors (TLF) are responsible for human serum resistance to non-infective subspecies of Trypanosoma brucei . TLF-1 is a High Density Lipoprotein particle (HDL) defined by the proteins Haptoglobin related protein (Hpr) and apolipoprotein L-1 (apoL-1). Hpr binds a hemoglobin dimer (Hb) analogously to the related circulating serum protein haptoglobin. TLF-2 is a lipid-poor complex containing apoL-1, Hpr, and IgM molecules. Both particles kill the parasites after lysosomal uptake via the haptoglobin-hemoglobin nutrient receptor. Hemoglobin-induced peroxidation has been suggested to play a role in the lytic mechanism of TLF-1. This research demonstrates that Hb binding to TLF-1 induces peroxidation of the lipids within the TLF-1 particle in vitro . Peroxidation of TLF-1 in human serum would create oxidation-specific epitopes for recognition and binding by innate oxidation-specific IgM molecules. IgM-bound TLF-1 might then mature into lipid-poor TLF-2, a hypothesis under investigation. Understanding immunological shifts in the TLF-1 and TLF-2 populations resulting from oxidative stress is important for elucidating the roles of these particles in the human innate immune response to parasites.

183 Repurposing human PDE4 inhibitors as a starting point for trypanosomal drug discovery

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Due to the paucity of drugs for sleeping sickness in the development pipeline, new approaches to drug discovery may be necessary, one such approach is target repurposing. Target repurposing is a way of circumventing one of the most significant bottlenecks in drug development i.e. identifying compounds with drug like properties. We are interested in human phosphodiesterase inhibitors as a starting point for the development of novel therapeutics for sleeping sickness. Multiple phosphodiesterase inhibitors have been extremely successful as drugs for a wide variety of diseases including respiratory disorders and erectile dysfunction. Double RNAi knockdown of the trypanosomal phosphodiesterases PDEB1 and PDEB2 in the bloodstream form render the parasites unable to divide correctly, which ultimately leads to death and as such are potential targets for the development of new therapeutics. Previously we reported our efforts investigating PDE4 and PDE5 inhibitors as potential leads for the development of new anti-trypanosomal compounds. PDE5 inhibitors showed little potency, however PDE4 inhibitors were more promising. We synthesised 340 compounds derived from piclamilast, GSK-256066 and cilomilast, which showed a narrow range of potencies, with IC50 and EC50 values in the micromalor to high nanomolar range. Despite efforts to explore multiple regions of the active site, including the kinetoplastid specific P-pocket, structure activity relationships have been difficult to discern. In order to improve our insight into how to make more potent inhibitors of TbrPDBEs we have characterized new protein constructs for use in crystallization and biophysical studies

184 Comparative Genomics of mRNA metabolism pathways in Eukaryotes

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The pathways of mRNA metabolism are central to gene expression. Maturation, translation and turnover are conserved pathways in eukaryotes and were present in the last common eukaryotic ancestor (LCEA). The regulatory mechanisms that control the pathways both at a general level, for example the overall rate of translation, or at a specific level, such as the entry of a specific mRNA into a degradation pathway, are more or less well conserved and have evolved features unique to each eukaryotic lineage. Here, the conservation of components associated with exon junction recognition, initiation of translation and mRNA degradation have been investigated using phylogenetic analysis of representatives of the 5 supergroups of eukaryotes providing a model for reconstruction of mRNA metabolism pathways in Excavates and Chromalveolates. The findings are: 1. Genes encoding a set of proteins involved in decapping, DCP1, DCP2 and PAT1 have been lost in Euglenids, including Kinetoplastids, but are present in all other groups of Eukarytotes. 2. Genes encoding translation initiation factors have undergone an independent expansion in Excavates but no loss or gain in Chromalveolates. 3. Apicomplexans are derived from a lineage resulting after endosymbiosis of a red alga. No trace of genes derived from red algal mRNA metabolism pathways were found in the Apicomplexan genomes. However, the absence of genes encoding exon junction components in the red algal genome may have resulted in selection pressure on the red alga eIF4A sequence.

185 New Tools for Studying Post-Transcriptional Regulation in Kinetoplastid Parasites

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Regulation of gene expression in kinetoplastid parasites occurs via post-transcriptional mechanisms that modulate mRNA turnover, translation rate, and/or post-translational protein stability. The application of systems-level approaches (e.g., RNA-seq and whole proteome profiling) to the study of global gene regulation in these parasites is becoming more common. We have developed two sets of molecular tools to simplify analysis of post-transcriptional regulation for candidates derived from global gene expression studies. Both techniques involve integration of reporter genes at the endogenous locus of the gene of interest in a manner that maintains the cognate 5'- and 3'- flanking sequences and are designed to fit into our recently described modular system for rapidly generating gene-targeting constructs. The first system utilizes dual luciferase reporters to study regulation at the mRNA and translational levels. We have used this system to analyze 15 candidate genes shown to be regulated by purine scarcity through our RNA-seq and comparative proteomic analyses of purine-starved Leishmania donovani promastigotes. Regulation of several of these genes was demonstrated to occur at the level of translation and/or mRNA abundance. In some instances, additional post-translational mechanisms were implicated. The second system permits translational and post-translational regulatory mechanisms to be distinguished via a single integrated reporter construct. This system utilizes the Thosea asigna virus 2A peptide sequence to elicit a cotranslational peptide cleavage between a genetically fused reporter and an epitope-tagged version of the gene of interest, coupling their translation but rendering the post-translational stability of the resultant polypeptides independent. Using this system, we have demonstrated that upregulation of the LdNT2 purine nucleoside transporter upon purine limitation is mediated through both translational and post-translational mechanisms.

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187 Mucin-Like Associated Surface Protein: Potential Vaccine Candidate against Chagas Disease

Serna, Carylinda (University of Texas at El Paso)

Trypanosoma cruzi is a flagellate protozoan that is the causative agent of Chagas disease. It currently affects 18-20 million people worldwide and has become an emerging disease throughout the world because of immigration and lack of screening. Therefore, there is an urgent need to develop a vaccine to be able to control this parasitic disease. In our study we have used a mucin-associated surface protein (MASP) (conjugated with keyhole limpet hemocyanin (KLH)) as synthetic peptide-based vaccine candidate. The study was done using 6-8 week female C3H/HeNHsd mice. 4 groups of mice were used: the experimental group was immunized with the peptide, the control group was given PBS; and for adjuvant controls, one group was given 0.9% aluminum hydroxide, and another KLH. The mice received 3 doses ten days apart. 12 after the last immunization antibody levels were measured and the mice were infected with a dose of 1 X 106 trypomastigotes Y strain. Parasitemia and survival were followed, in which increase survivability to immunized-infected mice was seen. In mice not infected, 4 weeks after the last immunization cytokine measurements were taken and showed to be increased in the immunized mice. We also evaluated the humoral response and detected specific anti-trypomastigote lytic antibody activity in immunized mice. 5 months after the last immunization, cellular mediated immunity response was detected in vaccinated mice by measuring CD8+ cytolytic activity. All together these results show that this synthetic peptide-based vaccine is able to control survivability by being able to prime both a humoral and cellular immunity.

188 Structure-function analysis of the novel chromosomal passenger complex in Trypanosoma brucei

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The chromosomal passenger complex (CPC), consisting of the kinase Aurora B and three evolutionarily conserved proteins, plays crucial roles in mitosis and cytokinesis in fungi and animals. In Trypanosoma brucei , the CPC is composed of the Aurora-like kinase TbAUK1 and two trypanosome-specific proteins, TbCPC1 and TbCPC2, which displays a unique subcellular localization during the cell cycle and regulates mitosis and cytokinesis in both procyclic and bloodstream forms. Despite the essential function of the CPC in trypanosomes, however, little is known about the regulation of TbAUK1 and the structure-function relationship of the CPC. Here, we investigate the effect of post-translational modifications of TbAUK1 on the activity and spatiotemporal control of TbAUK1 and demonstrate that autophosphorylation of two conserved threonine residues in the activation loop of the kinase domain contributes to TbAUK1 activation and function. Further, we find that TbAUK1 is modified by SUMO in vivo in trypanosomes and that SUMOylation of TbAUK1 is essential for TbAUK1 function. We also show that TbAUK1 undergoes proteasome-mediated degradation and that its degradation requires two destruction boxes, but not the KEN box, in the C-terminus of TbAUK1. Additionally, we determine the domains in individual CPC subunits that mediate the interactions among these subunits, and demonstrate that disruption of the interaction impairs the localization of TbAUK1 and TbCPC2, but not TbCPC1, suggesting that TbCPC1 likely mediates the targeting of the CPC to various subcellular locations during mitosis and cytokinesis. Together, our results demonstrate the requirement of post-translational modifications for TbAUK1 function and regulation and a crucial role of TbCPC1 in CPC localization.

189 The mitochondrial calcium uniporter of Trypanosoma brucei is essential for growth and infectivity

Huang, Guozhong (University of Georgia)*; Vercesi, Anibal (State University of Campinas); Docampo, Roberto (University of Georgia)*

Cytosolic Ca 2+ in trypanosomatids is maintained through the combined operation of distinct Ca 2+ transporting systems located in the plasma membrane, endoplasmic reticulum, mitochondria, and the acidic calcium stores known as acidocalcisomes. Mitochondrial calcium uptake in trypanosomatids is through a mitochondrial calcium uniporter (MCU) that has similar characteristics to the mammalian MCU and its identification had an important role in the discovery of the molecular nature of this uniporter in mammalian cells (Docampo and Lukes, Trends Parasitol. 28, 31-37, 2012). Localization studies in Trypanosoma brucei cell lines expressing MCU in its endogenous locus fused to an epitope tag or over-expressing a C-terminal GFP-tagged version of MCU revealed its co-localization with MitoTracker staining. MCU overexpression increased mitochondrial Ca 2+ accumulation in permeabilized T. brucei cells. Ablation of T. brucei MCU by RNAi markedly reduced mitochondrial Ca 2+ uptake without affecting their membrane potential, lowered ATP production, stimulated autophagosome formation, and produced marked defects in growth in vitro and infectivity in mice, revealing its essentiality in these parasites. The essentiality of MCU in trypanosomes was also confirmed by generation of conditional MCU knockout mutants of T. brucei bloodstream forms with loss of mitochondrial Ca 2+ uptake.

190 A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in *Leishmania donovani*.

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The salvage of purines by Leishmania is an obligatory process that impacts both cell viability and growth. Our previous studies demonstrated that purine starvation provokes significant morphological and metabolic changes, including the upregulation of permeases and salvage enzymes involved in purine acquisition and interconversion. To understand how Leishmania sense and adapt to changes in their purine environment, we have exploited purine pathway mutants. While wild type parasites are able to transport and convert any single purine nucleobase or nucleoside to fulfill their adenylate and guanylate nucleotide requirement, these purine pathway mutants have restricted purine requirements for growth. Thus, these mutants can be maintained in high levels of an extracellular purine that is non-permissive for sustained parasite replication. By culturing these purine pathway mutants in purines permissive or non-permissive for growth and subsequently monitoring for the upregulation of specific purine transporters and salvage enzymes — hallmarks for the adaptive response to purine starvation — we have determined that the adaptation to purine starvation arises from the surveillance of intracellular purine pools rather than from the direct sensing of the extracellular purine environment. Furthermore, our data suggest that the response to purine starvation is more profound when perturbations within the adenylate nucleotide pool are elicited rather than within the guanylate nucleotide pool, suggesting that adenylate nucleotide imbalances may be a primary trigger for the response to purine stress. Using a targeted metabolomic approach for profiling purine metabolites, we found that changes in intracellular purine pools occur within 2 h of purine removal from the media, and significantly, that at 24 h extreme changes in the levels of AMP, ADP, and ATP are evident.

191 Metabolic Reprogramming During Purine Stress in the Protozoan Pathogen Leishmania donovani

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The ability of Leishmania to survive in their insect or mammalian host is dependent upon an ability to sense and adapt to changes in the microenvironment. However, little is known about the molecular mechanisms underlying the parasite response to environmental changes, such as nutrient availability. To elucidate nutrient stress response pathways in Leishmania donovani, we have used purine starvation as the paradigm. The salvage of purines from the host milieu is obligatory for parasite replication; nevertheless, purine-starved parasites can persist in culture without supplementary purine for over 3 months, indicating that the response to purine starvation is robust and engenders parasite survival under conditions of extreme scarcity. To understand metabolic reprogramming during purine starvation we have employed global approaches. Whole proteome comparisons between purine-starved and purine-replete parasites over a 6-48 h span have revealed a temporal and coordinated response to purine starvation. Purine transporters and enzymes involved in acquisition at the cell surface are upregulated within a few hours of purine removal from the media, while other key purine salvage components are upregulated later in the time-course and more modestly. After 48 h, the proteome of purine-starved parasites is extensively remodeled, and adaptations to purine stress appear tailored to deal with both purine deprivation and general stress. To probe the molecular mechanisms affecting proteome remodeling in response to purine starvation, comparative RNA-seq analyses, qRT-PCR, and luciferase reporter assays were performed on purine-starved versus purine-replete parasites. While the regulation of a minority of proteins tracked with changes at the mRNA level, for many regulated proteins it appears that proteome remodeling during purine stress occurs primarily via translational and post-translational mechanisms.

192 BioID for trypanosomes - novel bilobe components identified by proximity to TbMORN1

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The complex and highly-ordered cytoskeleton of Trypanosoma brucei has been shown to play vital roles in its biology but remains difficult to study, in large part owing to the intractability of its constituent proteins. Existing methods of protein identification such as bioinformatic analysis, generation of monoclonal antibody panels, proteomics, affinity purification and yeast two-hybrid screens all have drawbacks. Proximity-dependent biotin identification (BioID) is a recently developed technique that allows forward screens for interaction partners and near neighbours in a native environment, and with no requirement for solubility in non-ionic detergent. As such, it is extremely well suited to the exploration of the cytoskeleton. In this project, BioID was adapted for use in T. brucei. The trypanosome bilobe, a discrete cytoskeletal structure with few known protein components, represented an excellent test subject. Use of the bilobe protein TbMORN1 as a probe resulted in the identification of seven new bilobe constituents and two new flagellum attachment zone proteins. This constitutes the first usage of BioID on a largely-uncharacterized structure, and demonstrates its utility in identifying new components of such a structure. This remarkable success validates BioID as a new tool for the study of unicellular Eukaryotes in particular, and the cytoskeleton in general.

193 Trypanosoma brucei RAP1 plays an important role in suppressing subtelomeric VSG associated gene conversion

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Trypanosoma brucei is a unicellular protozoan parasite that causes human African trypanosomiasis. T. brucei regularly switches to express a different surface antigen known as Variant Surface Glycoprotein (VSG), exclusively from subtelomeric VSG expression sites (ESs) in a monoallelic fashion. This antigenic variation mechanism is the key for establishing a persistent infection of T. brucei in its mammalian host. Two major pathways of VSG switching have been identified. One is called in-situ switching, where the active ES promoter is silenced while a previously silent ES promoter is activated. Second, homologous recombination (including gene conversion or crossover events) encompassing either the whole ES or a smaller fragment including VSG can lead to the replacement of the original active VSG gene with any new VSG gene. A combination of gene rearrangement and in-situ switch has also been observed in VSG switching events. Telomere is the nucleoprotein complex located at the ends of linear chromosomes and is required for chromosome stability. We have recently shown that TbRAP1, a telomere protein, is essential for complete silencing of ES-linked VSGs. Now we find that TbRAP1 also plays an important role in VSG switching regulation. A temporary depletion of TbRAP1 led to an increased VSG switching frequency, predominantly by VSG associated gene conversion.

194 Independent signalling mechanisms act to regulate life-cycle differentiation in Trypanosoma brucei

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Despite the detailed in silico analysis of the TriTryp kinome and phosphatome, the assembly of environmental signalling pathways in trypanosomes is almost entirely missing. One exception is a protein phosphatase cascade that regulates differentiation from bloodstream stumpy forms to vector adapted procyclic forms. In transmissible stumpy forms a tyrosine phosphatase (TbPTP1) prevents cells from differentiation until it is inactivated by the differentiation triggers citrate/cis-aconitate (CCA), whose uptake is controlled by the carboxylate transporter PAD proteins expressed on transmissible stumpy forms. Recently, we identified TbPIP39, a DxDxT phosphatase as a downstream regulator of this pathway and showed it is activated upon tyrosine-phosphorylation and negatively regulated by TbPTP1. Beside CCA, other differentiation triggers have been identified, such as mild acid, pronase and glucose depletion. To establish if these triggers operate through the CCA/ PAD/PTP1/PIP39 pathway, we measured differentiation of stumpy forms in response to each trigger when TbPIP39 was depleted by RNAi. We also monitored the activation of TbPIP39 using a specific antibody to detect its tyrosine phosphorylation after exposure to each trigger. This revealed that pronase mediated differentiation is not reduced upon TbPIP39 RNAi, unlike CCA and mild acid. Moreover, pronase did not result in TbPIP39 phosphorylation. This demonstrated that pronase stimulates differentiation independently of TbPIP39, and thus through a distinct signalling pathway to CCA and mild acid. In tsetse flies, however, midgut protease activity was not required for parasite differentiation. Our findings demonstrate that independent and redundant signalling pathways can operate to control parasite development in the tsetse fly.

195 The essential Leishmania major MAP kinase LmaMPK4 is involved in pH sensing and differentiation of metacyclic promastigotes

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We studied the role of the L. major MAP kinase LmaMPK4 using a novel knock out system based on the episome pXNG that renders transgenic parasites sensitive to the drug ganciclovir (GCV). LmaMPK4 null mutants established in pXNG-MPK4 transgenic parasites retained pXNG-MPK4 during negative selection despite the toxic effect of the drug, demonstrating an essential role of LmaMPK4 for promastigote viability in culture. Various LmaMPK4 mutants were created and tested for their ability to replace the pXNG-LmaMPK4 WT copy in a “plasmid shuffle” approach that combines negative selection with genetic complementation. First, we confirmed that the TXY motif, required for MAP kinase activation by higher order kinases, is essential for MPK4 function and parasite survive. Likewise, we found that a Leishmania-specific N-terminal sequence of 17 amino acids is required for promastigote viability. Surprisingly, MPK4 null mutants were viable expressing a putative dead mutant form of the kinase (MPK4-K59R). These parasites were normal in promastigote growth and morphology, but showed increased resistance to acidic pH in culture that was associated with a substantial increase in parasites that correspond to bona fide metacyclic parasites in morphology, surface glycolipid composition and density. Nevertheless, these parasites showed virulence attenuation in macrophage infection assays, especially at low multiplicity of infection. Kinase activity assays are currently performed in order to link this phenotype either to increased or attenuated MPK4-K59R activity. The genetic approaches presented here allow new insight into the function of an essential Leishmania protein kinase, which escapes classical knock out analyses due to the lethal null mutant phenotype. Our data reveal that MPK4 phospho-transferase activity may be involved in metacyclic differentiation and virulence.

196 RET1-DSS1 complex is required for gRNA maturation

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The U-insertion/deletion mRNA editing reactions are directed by guide RNAs (gRNAs). Mature 50-60 nt gRNAs are generated from ~800 nt precursors by 3' end nucleolytic processing and subsequent RET1-catalyzed uridylation. We previously demonstrated that TbRET1 repression in T. brucei leads to a loss of 3' oligo(U) tails and accumulation of gRNA precursors. The former observation is consistent with TbRET1's uridylyl transferase activity; the latter finding remained puzzling because TbRET1 lacks nucleolytic activity. Here, we show that TbRET1 forms a stoichiometric complex with a putative 3'-5' exonuclease TbDSS1 and several proteins without any discernible motifs, which we named TbRDS (TbRET1-TbDSS1) complex. In S. cerevisiae , DSS1 interacts with SUV3 helicase to form a mitochondrial degradosome. However, in T. brucei we found no evidence of stable DSS1-SUV3 interaction. Repression of TbDSS1 led to a loss of mature gRNAs and accumulation of ~800 nt gRNA precursors indicating that TbRET1 and TbDSS1 function in the same processing pathway. Furthermore, overexpression of enzymatically inactive DSS1 also triggered gRNA processing defects. In addition to minicircle-encoded gRNA precursors, TbRDS complex also targets maxicirlce-encoded messenger and ribosomal RNA precursors leading to generation of mature molecules. Collectively, our data indicate that nucleolytic processing of gRNA precursors by TbDSS1 and uridylation of mature gRNAs by TbRET1 are coupled by virtue of both enzymes being assembled into a stable TbRDS complex.

197 MITOCHONDRIAL CHAPERONE AND KDNA

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Mitochondrial chaperone Hsp70 (mtHsp70) takes part in many essential processes in the mitochondrion - folding of newly synthesized proteins and folding and degradation of damaged and denatured proteins. Moreover, this mitochondrial version of Hsp70 gained new functions such as in Fe-S cluster biogenesis and protein import into the organelle. Another poorly explored aspect of mtHsp70 is its association with mitochondrial DNA (mtDNA). This finding was never properly addressed in eukaryotes, while the bacterial homolog (DnaK) of mtHsp70 was proven to act in replication of both chromosomal and plasmid DNA, as well as the bacteriophage DNA. Trypanosoma brucei is a suitable model for studies of mtDNA, since there is only one large mitochondrion per cell and its mtDNA, represented by a dense huge network of circular DNA molecules - the kinetoplast (kDNA) is located close to the basal body of the flagellum and can be observed using light microscopy. Our preliminary data show that in cells depleted for mtHsp70, kDNA is getting smaller and eventually disappears completely. More detailed examination by electron microscopy revealed that the ultrastructure of kDNA is severely altered in almost 100% of cells ablated for mtHsp70. Sucrose gradient centrifugation revealed that a portion of mtHsp70 co-sediments with kDNA. Other putative functions of this mitochondrial chaperone and interacting proteins are under study.

198 Detecting and defining subdomains at the surface of African trypanosomes by proteomics

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To perform antigenic variation, the African trypanosome surface coat must be kept free of many essential invariant proteins. These are instead sequestered to the flagellar pocket, a specialised region of the surface membrane that is the sole site of endo/exocytosis. In previous work we and others defined morphological membrane domains and boundaries around the flagellar pocket, identified their association with the internal cytoskeleton, and described how nutrient macromolecules may gain access to the cell interior via a continuous channel linking the extracellular environment to the pocket lumen. However, there remains a paucity of data concerning the molecular composition of the flagellar pocket. This severely hampers understanding of flagellar pocket mechanisms, and also possible exploitation in drug and vaccine development. Using chemical derivatisation of surface membrane, tandem mass spectrometry, quantitative analysis and bioinformatic filters we describe a new surface proteome for bloodstream form Trypanosoma brucei. This set is enriched in GPI-anchored proteins, transmembrane proteins and annotations similar to known surface components. By creating a genetic toolkit for tagging membrane proteins from endogenous loci, we have localised several putative surface molecules of unknown function. Our results validate the surface membrane location of many novel components, and also show that individual proteins can access different combinations of cell body, flagellar and flagellar pocket membranes. We propose that T. brucei exhibits distinct domains on its surface with restricted diffusion between them. This paradigm has important implications for the function of the trypanosome cell surface.

199 Rab11 Regulates Glycosylphosphatidylinositol (GPI)-anchor Protein Trafficking to the Plasma Membrane by an Unconventional Pathway Involving the Contractile Vacuole of Trypanosoma cruzi

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Trypanosoma cruzi relies on protein secretion of glycosylphosphatidylinositol (GPI)-anchored proteins for invasion of host cells and establishment of infection. We identify T. cruzi Rab11 as a regulator of protein transport to the plasma membrane by an unconventional pathway involving the contractile vacuole complex (CVC). TcRab11 was expressed in T. cruzi tagged with green fluorescent protein (GFP) and shown to localize to the CVC. This localization was confirmed in the different life stages of the parasite using affinity-purified antibodies. We constructed Rab11:S21N (dominant negative, DN), and Rab11:Q66L (dominant positive, DP) mutants and transfected them into T. cruzi epimastigotes. DP mutants localized to the contractile vacuole, as expected. DN mutants localized to the cytosol. Epimastigotes expressing Rab11-GFP mutants were able to differentiate into metacyclic stages and infect host cells. T. cruzi GPI-anchored trans-sialidase (TS) and trypomastigote small surface antigen (TSSA) co-localized with Rab11 to the CVC before reaching the plasma membrane during transformation of intracellular amastigotes into trypomastigotes. Parasites expressing Rab11 DN mutants had morphological changes, prevented GPI-anchored proteins from reaching the plasma membrane, were more sensitive to osmotic stress, and were less infective as compared to wild type cells. Taking together these results reveal roles of TcRab11 in osmoregulation, trafficking of GPI-anchored proteins to the plasma membrane, and infection.

200 Simultaneous transcriptome profiling of Trypanosoma cruzi parasites and human host cells

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We have conducted a simultaneous transcriptome profiling of Trypanosoma cruzi (Y strain) and human host cells (dermal fibroblasts) during the course of an in vitro infection. Using RNA-Seq, we reconstructed the steady-state transcriptomes for the bloodstream and intracellular forms of the parasite at various time points post-infection. We have applied a rigorous statistical pipeline to remove batch effects in the datasets and used a combination of algorithms to conduct analyses. With the T. cruzi CL Brener genome used as reference, we have carried out the curation of gene models, the identification of novel ORFs, and the detection of the trans -splicing and polyadenylation sites at the single nucleotide level. De novo assembly of the transcriptome of Y strain was conducted in parallel and the comparison of our results to the reference genome of T. cruzi CL Brener strain reveals a high quality assembly which captures the majority of the transcripts in the pathogen, particularly for single or low-copy genes. Most interestingly, we have identified differential spliced-leader addition and polyadenylation events in the pathogen before and after the invasion of human host cells. These events may be associated with the regulation of mRNAs at the post-transcriptional level during the infection process. We have also investigated the gene expression patterns of T. cruzi at the chromosome level and at different time points and clustered genes with similar patterns. T. cruzi genes that are significantly regulated during the infection process may present new targets for drug development. Collectively, our transcriptomic analyses provide novel insights into the biology of T. cruzi including host-pathogen interactions that influence establishment and maintenance of intracellular infection in mammalian host cells.

201 On Being the right size -Trypanosoma brucei Sec16 and ER exit sites

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In the early secretory pathway protein and lipid cargo leave the ER in COPII secretory vesicles from a defined structure termed the ER exit site (ERES). These cargoes are subsequently processed in the Golgi and routed to their final destinations. The parasite Trypanosoma brucei represents a good model system for studying this pathway since it contains only a single ERES, Golgi and has a highly polarized architecture. How the parasite makes and exports proteins to the cell surface, and how it regulates the trafficking from the ER to the plasma membrane, is an important question. The efficiency of these processes in trypanosomes is integral to their phenomenal success as parasites. Using a bioinformatic approach we have identified a putative Sec16 in Trypanosoma brucei - a large peripheral scaffold protein involved in the formation of COPII vesicles, mediating transport between the ER and the Golgi. Phenotypic analyses of trypanosome cells following Sec16 depletion or overexpression revealed an intriguing link between secretory capacity and organelle size. Our data suggest that the size of the secretory organelles is just sufficient to cope with the cargo flux needed for optimal doubling time. Hence this organism is "stripped down", not just in terms of the types of secretory constituents but also in their amounts.

202 Inhibiting the essential FoF1-ATPase activity in the infectious form of Trypanosoma brucei

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The function of the essential mitochondrial (mt) FoF1-ATP synthase in Trypanosoma brucei differs between the insect and mammalian stages, producing ATP in the former while consuming ATP to maintain the mt membrane potential (mt ??) in the absence of a cytochrome-mediated respiratory chain in the latter. Importantly, the FoF1-ATPase activity can be specifically inhibited by the natural inhibitory protein, IF1, which has an expression profile that is only detected in the insect stage of the parasite. When an ectopic T. brucei IF1 (TbIF1) is over-expressed in the infectious mammalian stage, the mt ?? collapses, leading to the death of the parasite. Crucially, this in vivo expression of TbIF1 inhibits only the ATPase activity without interfering with ATP synthesis. Additional characterization studies using recombinant TbIF1 indicate that this protein has a slightly acidic pH optimum for binding to the purified Tb-F1-ATPase, which correlates to the physiological pH of the mt matrix when the mt ?? decreases and triggers ATP hydrolysis. Interestingly, while the amino acid sequence of TbIF1 is moderately similar to the IF1 peptide found in higher eukaryotes, the bovine protein does not inhibit the Tb-F1-ATPase and vice versa. This exciting result presumably reflects key differences in the TbIF1 protein sequence and structural regions that interact with Tb-F1-ATPase. Therefore, a series of truncated and mutated TbIF1 proteins were prepared to map the essential residues and motifs necessary for the interaction between TbIF1 and Tb-F1-ATPase. Our results suggest that the T. brucei FoF1-ATPase is an attractive drug target because it is possible to specifically inhibit the activity of the FoF1-ATPase, an essential activity for the parasite but not for the host.

203 The cooperative roles of PHO80-like cyclins in regulating the G1/S transition and posterior cytoskeletal morphogenesis in Trypanosoma brucei

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Cyclins and cyclin-dependent kinases (CDKs) represent the fundamental, crucial regulators of the cell division cycle in eukaryotes. Trypanosoma brucei expresses a greatly expanded repertoire of cyclins and C DK- r elated k inases (CRKs). However, how these cyclins and CRKs cooperate to regulate cell cycle progression in trypanosomes remains poorly understood. Here, we carry out directional yeast two-hybrid assays to identify the interactions between the 10 cyclins and the 11 CRKs and detect a total of 26 cyclin-CRK pairs, among which 20 cyclin-CRK pairs are new. These results suggest that the cyclin-CRK system in trypanosomes is more complex than previously thought. Our current efforts are focused on four PHO80-like cyclins, CYC2, CYC4, CYC5, and CYC7, and their physical and functional interactions with CRK1. RNAi silencing of each of the four cyclins and CRK1 unanimously arrests the procyclic trypanosome cells at the G1 phase, suggesting their essential roles in promoting the G1/S transition. Additionally, CYC2-, CYC7-, and CRK1-deficient cells possess an elongated posterior, and knockdown of CYC7 or CRK1 also produces cells with branched posteriors. Posterior elongation is likely attributed to the extension of the subpellicular microtubule corset toward the posterior tip of the cell, indicating that CYC2 and CYC7, together with CRK1, may play an additional role in coupling of posterior cytoskeletal morphogenesis to the G1/S transition. Further, the four cyclins display distinct subcellular localizations and half-lives, implying that they likely undergo distinct regulation. Altogether, our results demonstrate the involvement of four CRK1-associated cyclins, CYC2, CYC4, CYC5, and CYC7, in promoting the G1/S transition and the requirement of CYC2 and CYC7 in maintaining cell morphology during the G1/S transition.

204 Distinct PPR proteins are responsible for coupling of mRNA editing, polyadenylation and translation in mitochondria of trypanosomes

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The majority of trypanosomal mitochondrial pre-mRNAs undergo massive uridine insertion/ deletion editing which creates open reading frames. However, our recent findings indicated that pre- and post-editing processing steps are also required to produce translation-competent mRNAs. Pre-editing addition of short 3' A-tails exerts no influence on unedited pre-mRNA stability, but stabilizes transcripts that are edited beyond few initial sites. The post-editing extension of A-tails into A/U heteropolymers by KPAP1 poly(A) polymerase and RET1 TUTase commits fully-edited mRNAs to translation. To identify factors responsible for coupling of editing, polyadenylation and translation we built a comprehensive protein interactions network of respective machineries. The ensuing RNAi screen distinguished several pentatricopeptide repeat-containing (PPR) RNA binding proteins acting to: 1) stabilize mRNA prior to polyadenylation (PPR15); 2) block premature mRNA uridylation (PPR14) and 3) induce transcript-specific adenylation/ uridylation (PPR26 and PPR28). PPR15 is similar to the kinetoplast polyadenylation/ uridylation factor 1 (KPAF1) and is an integral subunit of the polyadenylation complex. PPR14 associates transiently with both KPAP1 and RET1, and is likely to be a membrane-anchored protein. Unexpectedly, PPR26 and PPR28 are stably associated with small ribosomal subunit (SSU) and the polyadenylation complex. Investigation of these PPR proteins suggested that pre-mRNA is initially stabilized by PPR15 binding which may also recruit poly(A) polymerase resulting in A-tail addition. Initiation of RNA editing at the 3' region displaces PPR15 leaving A-tail as the main cis-acting stability element. We propose that SSU-associated PPR proteins, such as PPR26 and PPR28, recognize specific RNA sequences and stimulate transcript-selective A/U-tailing thereby committing mRNA for translation. Finally, PPR14 is likely to be actively disengaged from fully-edited mRNA to allow for A/U-tail addition.

205 High-throughput screening of a kinase-targeted library at the GlaxoSmithKline OpenLab: Identification and characterization of thousands of anti-trypanosomal hit compounds and opportunities for “open-source” lead optimization.

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Noting the prominent position kinases play as potential therapeutic targets in trypanosomes, we performed a high-throughput screening campaign that assessed 46,688 kinase-targeted compounds from GlaxoSmithKline’s corporate collection for their growth inhibitory properties against Trypanosoma brucei brucei cultures. This resulted in 6920 compounds that inhibited parasite growth >50% at 4 μM concentrations. Following confirmation screens and structural clustering, 4,621 compounds were tested in dose-response assays against T brucei and counterscreened for host cell toxicity (HepG2 cells). This resulted in identification of 797 compounds that had better than 1 μM potency against T brucei cells, with at least 100-fold selectivity over HepG2. Of these, we selected compounds that showed submicromolar activity at 18 hours of incubation or predicted CNS penetration, an exercise that culminated in ~300 compounds that could be grouped into 23 structural clusters. We will describe our ongoing efforts to pursue these results, including characterization for human kinase selectivity, drug metabolism properties, rate of action, and physicochemical properties, and will outline our plans to make these data available for pursuit by the wider neglected tropical disease community.

206 Immunolocalization of the virulence factor NTPDase-1 in Trypanossoma cruzi

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Trypanosoma cruzi is the causative agent of Chagas disease. T. cruzi Nucleoside Triphosphate Diphosphohydrolase 1(TcNTPDase-1) was previously showed as a facilitator of infection and a virulence factor highlighted it as a good target to rational drug design. In this work we produced TcNTPDase-1 in bacterial system and evaluated its cellular localization. For this, we done Western blot analysis of TcNTPDase-1 expression in epimastigote and used confocal and transmission electron microscopy. The use of specific polyclonal antibodies allowed us to confirm the presence of TcNTPDase-1 at the surface of parasites by confocal and electron microscopy. In addition, electron microscopy revealed that TcNTPDase-1 was also found in the flagellum, flagellum insertion region, kinetoplast, nucleus and vesicles similar to reservosomes. The presence of this enzyme in the flagellum insertion region and reservosomes suggests a role for it in nutrient acquisition by epimastigotes because they are a replicative form of the parasite, which has a metabolism that requires high levels of purines and derivatives to replicate DNA, transcribe RNA and execute other purine-dependent pathways and the spread profile suggests that it may be involved in other biological process. The reservosomal localization of the enzyme has been confirmed by a recent and specific reservosome proteomic study (Sant'Anna et al., 2009). These data reinforce the involvement of TcNTPDase-1 in the metabolic nutrition of epimastigotes. Our data open new frontiers to future investigations that could be used in the rational design of drugs to treat Chagas disease based in the unknown functions of NTPDase-1 related to its ubiquitous expression. We would like to thank the Núcleo de Microscopia e Microanálise da Universidade Federal de Viçosa (UFV), Fundação Coordenação de Aperfeicoamento de Pessoal de Nivel Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq), Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG), Instituto Nacional de Biotecnologia Estrutural e Química Medicinal em Doenças Infecciosas and Universidade Federal de Viçosa

207 Insights into expression of GPEET procyclin during differentiation from early to late procyclic form T. brucei

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EP and GPEET procyclin are the major surface proteins of procyclic forms of T. brucei . Expression of the two proteins is differentially regulated - GPEET is expressed exclusively by early procyclic forms while EP is expressed by both early and late procyclic forms in tsetse. EP and GPEET share three elements in their 3' UTRs that regulate mRNA stability and translation. An additional element known as the glycerol-responsive element (GRE), is unique to GPEET. Although GPEET is transcribed in late procyclic forms, the GRE acts as an RNA destabilising element at this stage. We performed a detailed analysis of the differentiation from early to late procyclic form parasites in culture and found that downregulation of GPEET is influenced by parasite density, with dense cultures repressing GPEET more rapidly than dilute cultures. Inhibition of transcription by Actinomycin D revealed that GPEET mRNA was surprisingly stable in early forms (t 1/2 > 6h) and could even be detected 6h after the addtion of Actinomycin D to late procyclic forms. Mapping of GPEET mRNA 3' ends by an RT-PCR-based approach showed shortening of poly(A)-tails after 6h in both early and late procyclic forms. Samples from late procyclic forms were enriched in non-polyadenylated cDNAs with extended 3' UTRs. We also obtained cDNAs with shortened 3' UTRs that lacked poly(A)-tails, as well as 3' UTRs that harboured non-templated oligo(U)-tails. We postulate that the short untailed mRNA species correspond to endonucleolytic cleavage products and secondary degradation products, and that the oligo(U)-tails might represent signals for these processing events.

208 The bi-lobe associated TbLRRP1 is a RanGTPase activating protein

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T.brucei flagellum runs along the cell body to which it adheres via the Flagellum Attachment Zone (FAZ). Located at the base of both the FAZ and the flagellum is a bi-lobed structure important for Golgi duplication and segregation. Previously our laboratory identified TbLRRP1 as a component of this bilobe. Upon TbLRRP1 depletion, the new FAZ assembly is inhibited, raising questions about new functions for the bilobe (Zhou et al., 2010). In this study, a yeast two hybrid screening identified an interaction between TbLRRP1 and a putative RanBP1-like protein :TbRB1L. Both TbLRRP1 and RanBPL1 bound to Ran GTPase, a key regulator of the nucleo-cytoplasmic transport, mitotic spindle formation and nuclear envelope reassembly, forming a functional complex that was capable of hydrolyzing RanGTP to RanGDP. The RanGAP activity of TbLRRP1 in vivo was further confirmed by inducible TbLRRP1 depletion, which disrupted the nuclear localization of YFP-TbRan as well as the nuclear/cytoplasmic gradient of RanGTP/RanGDP. Most remarkably, as has been shown previously (Zhou et al., 2010), depletion of TbLRRP1 had little effect on mitosis. This was distinct to cells lacking TbRan that exhibited a rapid block on nuclear division. These results indicate that whereas TbLRRP1 can function as a RanGAP in maintaining RanGTP/RanGDP gradient, its primary role is on FAZ assembly, supporting flagellum functions.

209 p24 transmembrane proteins regulate early secretory trafficking in African trypanosomes

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The critical virulence factor of Trypanosoma brucei , the causative agent of African sleeping sickness, is the Variant Surface Glycoprotein (VSG). VSG originates in the lumen of the endoplasmic reticulum (ER) and is trafficked through the flagellar pocket and onto the cell surface, where proper surface localization of VSG is required for the pathogenesis of T. brucei . VSG is trafficked out of the ER in vesicles whose budding is driven by the assembly of cytoplasmic coat complex (COPII) subunits on the ER membrane. Interestingly, VSG is sorted into only a subset of COPII vesicles, those composed of TbSec23.2 & TbSec24.1 subunits, (but not those expressing TbSec23.1 & TbSec24.2). This presents an interesting topology problem: How are GPI-VSG molecules (lumenal facing) selectively included into only a subset of ER vesicles, when COPII components are cytoplasmic? We hypothesized that a member of the p24 family of transmembrane proteins serves simultaneously as both a lumenal receptor for VSG and a cytoplasmic anchor for specific COPII components. In other systems, p24 proteins regulate COP-mediated vesicular trafficking between the ER and the Golgi, and in yeast, they play a role in GPI-anchor recognition during sorting from the ER. The T. brucei genome encodes eight predicted members of the p24 family: TbERP1-8 , and the streamlined architecture of the Trypanosome secretory system provides a unique opportunity to determine the roles of these p24 proteins in the selective inclusion of secretory cargo (VSG in particular) into COPII vesicles exiting the ER. Here we investigate the roles of these putative cargo receptors in selective protein trafficking and organelle integrity in the early T. brucei secretory pathway.

210 RNA Polymerase II Transcription of the Serum Resistance Associated Protein gene containing Expression Site in Trypanosoma brucei rhodesiense

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Trypanosoma brucei brucei cannot establish an infection in humans because of susceptibility to trypanosome lytic factor (TLF-1), a minor subclass of high-density lipoproteins (HDLs). Trypanosoma brucei rhodesiense, the causative agent of acute human sleeping sickness (HAT), however, can infect humans because it has evolved resistance through expression of the Serum Resistance Associated protein (SRA). The SRA gene was found to be exclusively located in a truncated Bloodstream Expression Site (BES) (Xong et al. 1998). RNA Polymerase I transcription of the Variant Surface Glycoprotein gene (VSG) occurs exclusively from a BES, from which only one is actively transcribed at the time. Transcriptional analysis of cells which have undergone ES switching shows that SRA can not only be transcribed by RNA Polymerase I (as part of the actively transcribed BES), but also by RNA Polymerase II (independently of the actively transcribed BES). This demonstrates that in-situ switching of ESs can occur in T. b. rhodesiense and therefore expand the repertoire of VSG switching mechanisms in antigenic variation in the human host without loss of SRA expression. During the initial phase of an infection, to avoid destruction by the host's immune system, Metacyclic Form trypanosomes (MF) express the Metacyclic Variant Surface Glycoprotein (mVSG) exclusively from a mono-cistronic Metacyclic Expression Site (MES). During this life cycle stage all BESs are transcriptionally inactive. BES independent RNA Polymerase II transcription of SRA may therefor allow for survival against killing by TLF-1. Upon differentiating from bloodstream form (BF) into the procyclic form (PF) BESs become transcriptionally inactive. We observe however continuous expression of the SRA gene, which could explain how MF trypanosomes can circumvent killing by TLF-1.

211 Action of Trypanosome Lytic Factor Against Leishmania major

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Trypanosome Lytic Factor (TLF) is approximately one percent of high density lipoprotein and provides innate immunity to human hosts by protecting against most species of African Trypanosomes. The lytic protein is activated in low pH, thus indicating potential action against pathogens localizing to acidic environments. TLF has been shown to protect against Leishmania, a kinetoplastid parasite, which replicates in the phagolysosomes of macrophages. TLF increases opsinization of the parasite for uptake by macrophages, where it is activated in the acidified parasitophorous vacuole. TLF is able to reduce parasite load by forming pores in promastigote-stage parasites inside the phagolysosome without activating the macrophage. The parasite is no longer susceptible to TLF, however, once it transforms to the amastigote stage inside the mammalian host. We show that these pores are formed directly in the plasma membrane of the parasite at low pH such as that within the macrophage phagolysosome, resulting in dissipation of membrane potential and swelling of the parasite due to osmotic influx of water. Promastigote mutants lacking components of the surface coat, leading to a surface phenotype similar to the amastigote stage, are also shown to have variable susceptibility to TLF-mediated lysis ex vivo . The data presented propose a novel target for TLF and potential mechanism for lysis, indicating a potential, general anti-microbial function for this innate immune factor.

212 Transforming a Parasite into an Antigen Display Platform

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Trypanosoma brucei is a protozoan parasite whose surface coat consists of a dense and repetitive array of a single highly immunogenic Variant Surface Glycoprotein (VSG). While the organism has evolved multiple mechanisms of immune evasion, the immune response to the VSG coat is robust, long lasting, and almost exclusively antibody-mediated. This immune response can be exploited by engineering recombinant VSG coats to utilize T. brucei as an antigen display platform. We have shown previously that by inserting foreign peptides into VSG surface loops, we can generate antibodies specific to these peptides. We have now expanded the versatility of this system by covalently linking diverse antigens (such as proteins and small molecules) to engineered surface VSGs through the action of the enzyme Sortase A. We are currently using this technology to generate antibodies to varied targets and to study the immune response to T. brucei.

213 Identification and functional analysis of a GPR89 homologue that promotes the development of stumpy forms in Trypanosoma brucei

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G protein-coupled receptors are seven transmembrane domain (TM) proteins that are located in the plasma membrane and transduce signals through their interactions with extracellular small molecule ligands and G protein dependent and independent signaling cascades that allow cells to respond to changes within their environment. Although trypanosomes are conventionally described as lacking GPCRs, we have identified a T. brucei protein related to the GPR89 family members that have been implicated in G-protein signaling in plants and acidification of the Golgi in mammals. The T.brucei protein has eight predicted TM and unlike the human protein, localizes to the plasma membrane. Western blot analysis shows that in pleomorphic cells (i.e. capable of generating slender and stumpy forms), the protein is expressed in slender forms but the levels rapidly decrease as cells differentiate into intermediate and stumpy forms. In pleomorphic parasites inducible overexpression of the full length and a C-terminally tagged version of the protein drives premature trypanosome differentiation in vitro and in vivo, generating stumpy cells at lower cell density compared to uninduced or the parental cell line. When overexpressed, the protein is rapidly degraded in what it seems to be an ubiquitin-dependent process, similar to the desensitization of GPCRs in other organisms. Our experiments indicate that TbGPR89 might be expressed on slender forms as a sensor of an external stimulus thus initiating stumpy formation.

214 DNA double strand break repair in Trypanosoma brucei: reaction kinetics and factors

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Our current view of DNA double strand break (DSB) repair in Trypanosoma brucei is that homologous recombination appears to be the major pathway, with an end-joining reaction based on sequence microhomology operating in the background. However, the relative efficiencies of the reactions have been little explored, and their relative contribution to repair only examined in a limited range of settings. Taking advantage of a meganuclease I-SceI system developed by D. Horn and colleagues, which allows targeted generation of DNA DSBs, we have investigated the spatial and temporal localization of RAD51, the key player in homologous recombination, relative to a number of other factors involved in DNA repair in T. brucei, and have used novel PCR assays to explore the dynamics of DSB repair. We reveal surprising complexity in the timing of break repair, and show that BRCA2 and RAD50 appear to localise to DSBs rapidly, well before RAD51. In addition, using co-immunoprecipitation, we have uncovered a novel BRCA2 interacting factor: a putative DNA helicase that has emerged as a key factor in genome maintenance in some eukaryotes, though its precise functions remain unclear. We will describe the role of this factor in DNA repair and BRCA2 function.

215 Mechanism of flagellum construction and maintenance

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Cilia and flagella are cylindrical organelles that protrude at the surface of numerous eukaryotes including most human cells. They are composed, from base to tip, of the basal body (9 triplet microtubules), the transition zone (TZ, 9 doublet microtubules) and the axoneme (9 doublet microtubules ± 2 central microtubules). New flagellar sub-units are added at the distal tip by intraflagellar transport (IFT), a dynamic process where IFT motors drag IFT particles in both anterograde and retrograde directions. IFTs concentrate in a pool at the base of the flagellum, whose localization depends on the organism. In Trypanosomatidae, immunofluorescence assays reveal the association of this pool (detected by an anti-IFT172 antibody) to the TZ, split apart from the cytoplasm by the transitional fibres (TF, detected by YL1/2 antibody). In Trypanosoma brucei , we undertook to investigate about the role of the TZ in flagellum formation and maintenance of IFT in mature flagella, by deciphering the role of RP2, a candidate protein located at the TFs [Stephan et al. , Traffic 2007]. We constructed a strain impaired in RP2 production by tetracycline-inducible RNAi and obtained the expected phenotype: parasite growth was affected from the third day of induction. In parallel, immunofluorescence assays revealed that flagellum length was reduced and that IFT172 labelling was modified. Diminution of IFT concentration at the flagellum base suggests that RP2 is involved in IFT recruitment during flagellum formation and/or RP2 takes part in a ciliary gate that hinders IFT leaking towards the cytoplasm. We are currently studying these hypotheses by live microscopy using fluorescent fusions of RP2 and IFT proteins, dissecting the molecular mechanisms involved in flagellum construction and homeostasis.

216 Investigating the role of the *Leishmania (L.) major* HASPs and SHERP genes during metacyclogenesis in the sand fly vectors *Phlebotomus papatasi* and *P. duboscqi*

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During metacyclogenesis in the Leishmania life cycle, procyclic promastigotes differentiate into mammalian-infective metacyclic parasites within the sand fly vector. We have shown previously that parasites deleted for the L. (L.) major LmcDNA16 locus (a region of chromosome 23 that codes for the stage-regulated HASP and SHERP proteins) do not complete metacyclogenesis in the sand fly midgut, although metacyclic-like stages can be generated in in vitro culture (Sádlová et al. Cell. Micro.2010, 12, 1765-79). To determine the contribution of individual genes in the locus to this phenotype, we have generated a range of mutants in which target HASP and SHERP genes are reintroduced either individually or in combination into their original genomic locations within the L. (L.) major cDNA16 double deletion mutant. All replacement strains have been characterized with respect to their gene copy number, correct gene integration and stage-regulated protein expression, passaged through susceptible mice and then used to infect the L. (L.) major specific sand fly vectors, P. papatasi and P. duboscqi. Following infection, the progress of parasite metacyclogenesis has been monitored over twelve days by midgut dissection and microscopy. Metacyclogenesis was not fully recovered in any of the replacement mutants tested. Surprisingly, HASPB protein expression could not be detected in the replacement mutants within the sand fly midgut either, although HASPB protein was readily detected when the same parasite lines were cultured in vitro. The same was true for SHERP, although in situ expression was recovered in the presence of a HASPB gene, which itself did not expressed detectable HASPB protein levels. These observations suggest a requirement for an as-yet-unidentified regulatory component for HASPB expression within the sand fly midgut which is not required in culture.

217 Homologue of a human mitochondrial deadenylase is a cytosolic ribonuclease in T. brucei

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mRNA decay, which normally initiates with deadenylation, is an especially vital component of gene regulation in trypanosomes. In a search for enzymes that may contribute to mRNA decay in T. brucei, we identified an enzyme with high sequence similarity to the human deadenylase PDE12 that we term EEP1 (for exo/endo/phosphatase domain containing protein 1). Like PDE12, recombinant EEP1 exhibits exoribonuclease activity in the 3' – 5' direction. EEP1 is active on numerous RNA sequences, although RNAs ending in poly(A) are preferentially degraded. In contrast, EEP1 is completely inactive on RNAs ending in uridine homopolymers. EEP1 is not a functional homologue of PDE12, as PDE12 is a mitochondrial deadenylase, whereas subcellular fractionation and immunofluorescence experiments demonstrate that EEP1 is cytosolic. EEP1 depletion by RNAi results in a slight growth inhibition in PF cells. To identify RNAs that are stabilized in the EEP1-depleted cells, we analyzed RNA from uninduced and induced EEP1 RNAi cells by Illumina sequencing. We identified a small set of tRNAs and snoRNAs, noncoding RNAs for which adenylation may play a role in decay, as well as a small set of mRNAs that change in abundance upon EEP1 depletion. Previous genome wide studies may provide additional clues to EEP1 function. RIT-seq analysis suggested that EEP1 depletion results in a gain of fitness during bloodstream to procyclic differentiation. Additionally, EEP1 is among the mRNAs whose abundance is altered upon expression of a mutant form of DHH-1, a helicase associated with P-bodies. mRNAs that were affected in this analysis were often developmentally regulated, leading us to ask if the primary role of EEP1 might be in developmental regulation and/or differentiation. To this end, we are depleting and overexpressing EEP1 in BF cells and will examine these cells' ability to differentiate.

218 TbISWI and NLP are part of a novel ISWI complex in T. brucei

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T. brucei has a vast repertoire of Variant Surface Glycoprotein (VSG) genes, of which one is expressed at a time from one of about 15 VSG expression sites. It is a mystery how singular expression of VSG is achieved. Chromatin remodelers including TbISWI play a key role in VSG expression site control. TbISWI is involved in repression of VSG expression sites, and is highly enriched at Pol II strand switch regions (SSR). Different ISWI complexes have been found in most if not all eukaryotes, and these can have different roles depending on their composition. In order to shed light on the TbISWI complex(es) of T. brucei, we performed protein affinity purification on PTP-epitope tagged TbISWI. We identified three TbISWI protein partners: a 70 kDa protein with four RCC1 domains (regulator of chromosome condensation 1) and a 50 kDa protein with a FYRC domain (found in several chromatin-associated proteins). Last, and surprisingly, the previously characterised nucleoplasmin-like protein NLP was found to be part of an ISWI complex. We confirmed these results using PTP-epitope tagged NLP instead of TbISWI. Using co-immunoprecipitation we confirmed that these four proteins form at least one TbISWI complex. We are currently investigating the localisation of these TbISWI partner proteins with ChIP-qPCR to determine if there are multiple ISWI (sub)-complexes in T. brucei. We therefore show that T. brucei has an unusual ISWI complex. None of the identified TbISWI partners show homology with known ISWI partners, although all three have chromatin-associated motifs. We are currently investigating which areas of the T. brucei genome are particularly affected by TbISWI-mediated chromatin remodeling.

219 Trypanosoma brucei Polo-like kinase: function and regulation

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Polo-like kinases (PLK) play multiple roles across the cell cycle, particularly during mitosis, and are highly conserved among model eukaryotes. This is in contrast to T. brucei PLK (TbPLK) where studies characterising its function have found no evidence for a role in mitotic regulation. Instead, TbPLK is essential for Golgi biogenesis, basal body duplication and kinetoplast DNA segregation in procyclic form (PCF) parasites and for furrow ingression during cytokinesis in the bloodstream form (BSF). However, exactly how the activity of TbPLK is regulated is not known. TbPLK has a well conserved N-terminal Ser/Thr kinase domain, complete with the regulatory T-loop, and a C-terminal Polo-box domain (PBD). In other eukaryotic organisms, the PBD targets PLK towards its substrates, a His-Lys pincer in the PBD is essential for substrate binding and the phosphorylation of a conserved threonine residue in the T-loop of the kinase domain is essential for PLK kinase activity. Previously, we have shown that ectopic overexpression of TbPLK in PCF cells results in cell cycle defects, while overexpression of a kinase dead variant does not. Using this overexpression assay, the modes of PLK regulation in T. brucei were investigated showing that T198 phosphorylation is essential for TbPLK kinase activity, in agreement with published data (Yu et al, 2012), while the PBD is not. Since a second T-loop threonine residue (T202) has been shown to be important for TbPLK kinase activity, experiments are in progress to determine the relative importance of T198 compared to T202 in addition to those investigating the regulation of TbPLK kinase activity in BSF trypanosomes.

220 Identification and Comparison of polymorphisms in the Trypanosoma cruzi Complement Regulatory Protein (Tc-CRP) encoding gene from different parasite strains

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The Trypanosoma cruzi Complement Regulatory Protein (Tc-CRP), a major epitope that induce lytic antibodies during T. cruzi infection, is a glycoprotein that binds C3b and C4b components of the complement system, allowing the parasite to evade the host immune response. In recent decades, biochemical and molecular studies classified Tc-CRP as a family member of the trans-sialidase and evaluated its application as a molecular marker in serological tests for diagnosis before and after treatment. However, to date, all studies were performed using only the T. cruzi Y strain, prompting the necessity to assess the presence and expression of Tc-CRP in other strains. So, this study aimed the evaluation of polymorphisms present in the Tc-CRP encoding gene in different T. cruzi strains belonging to groups I to VI, isolated from various geographic regions. To confirm the presence of the Tc-CRP coding gene in different parasite strains we designed three set of primers (CRP PRÉ, CRP-1 and CRP-2) which allowed the amplification of fragments which covered the complete gene and were used as templates in the sequencing experiments. The PCR using the designed primers (CRP PRÉ, CRP-1 and CRP-2), which amplified fragments of approximately 466bp, 1486bp and 1569bp, respectively, allowed the amplification of the UTR region, beyond the carboxyl and amino-terminal regions of the protein open reading frame, respectively. In conclusion, this study confirmed the presence of the Tc-CRP gene in all evaluated strains and demonstrated the molecular diversity among them, suggesting an explanation for different levels of lytic antibodies induction observed in experimental models as well as in chagasic patients.

221 Investigating T. brucei oligosaccharyltransferase STT3A and STT3B sequon specificity in vivo.

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T. brucei contain multiple essential proteins that are N-glycosylated, a posttranslational modification that modulates folding, stability and activity. Thus, transferring N-glycans to nascent proteins is crucial for parasite survival and a potential therapeutic target. Unlike the eight subunit complex in mammals, the oligosaccharyltransferase (OST) in T. brucei has been suggested to function with a single catalytic subunit, STT3. However, the parasite has three slightly different STT3 genes - TbSTT3A, TbSTT3B and TbSTT3C - in a tandem array. Only TbSTT3A and TbSTT3B have been seen expressed, in both the bloodstream and procyclic forms. Furthermore, T. brucei uses two precursor N-glycans, Man5GlcNAc2 and Man9GlcNAc2, unlike the single Glc3Man9GlcNAc2 precursor used in mammals. While transferring glycans to the same sequon, TbSTT3A is responsible for co-translational transfer of the biantennary Man5GlcNAc2, whereas TbSTT3B catalyses post translational transfer of triantennary Man9GlcNAc2 to the remaining sterically accessible sequons. The existence of two enzymes glycosylating the same sequon necessitates an additional determining factor for N-glycan transfer. Through proteomic and bioinformatic studies, the current hypothesis states that TbSTT3A transfers N-glycans to sequons in an acidic amino acid environment, while TbSTT3B transfers N-glycans to sequons in both neutral and basic amino acid surroundings. Using a panel of cell lines expressing the N-linked glycosylation sequon in different amino acid environments, we have established an in vivo assay to map the variation in N-glycan attachment (i.e. biantennary or triantennary glycans) directed by different sequon environments. This assay allows us to validate the current hypothesis and provide greater understanding of TbSTT3A and TbSTT3B sequon specificity in N-linked glycosylation.

222 An endossomal eIF2alpha kinase is activated by phosphorylation during reservosomes depletion in Trypanosoma cruzi

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Trypanosoma cruzi, the causative agent of Chagas' disease, faces different environmental conditions during its life cycle, such as starvation and alterations in temperature and pH, which requires the activation of specific metabolic pathways to its survival. Among those, regulation of translation initiation through the phosphorylation of the alpha subunit of translation initiation factor 2 (eIF2alpha) has been demonstrated to play a key role (Tonelli et al. 2011, PlosOne 6:e27904). Three eIF2a putative kinases are present in T. cruzi, TceIF2-K1, K2 and K3. Here we show that TceIF2-K2 is distributed in specialized endosomal organelles called reservosomes of proliferating epimastigote form. Exposition of epimastigotes to low nutrients triggers differentiation into metacyclic-trypomastigotes that shown low protein synthesis. We observed that during metacyclogenesis reservosomes are consumed with TceIF2-K2 dispersion through small vesicles in the cytosol. Simultaneously, TceIF2-K2 becomes phosphorylated. These results suggest that reservosomes consumption triggers the kinase activation by phosphorylation, arresting protein synthesis in the metacyclic-trypomastigotes. Support by FAPESP, CNPq

223 TbTim62: A novel component of the mitochondrial inner membrane protein translocase in Trypanosoma brucei.

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Ujjal K Singha and Minu Chaudhuri*, Microbiology and Immunology, Meharry Medical College, Nashville, TN 37208. *Corresponding Author: mchaudhuri@mmc.edu Translocases of Mitochondrial Inner Membrane are multi-protein complexes widely conserved in fungi to mammals. However similar protein complexes are not well delineated in kinetoplastid parasites, like Trypanosoma brucei. Recently, we characterized Tim17 in T. brucei (TbTim17), the single homolog of Tim17/22/23 protein family. TbTim17 is essential for cell survival and mitochondrial protein import. TbTim17 is present in a protein complex of about 1,100 kDa, which is much larger than the TIM complexes found in other eukaryotes. Purification of the TbTim17-containing protein complex from the mitochondrial membrane of T. brucei by tandem affinity chromatography (TAP) revealed that TbTim17 associates with several unique T. brucei mitochondrial proteins. One of these novel proteins, TbTim62, was further characterized to elucidate its role in mitochondrial protein import. TbTim62 is localized in the mitochondrial inner membrane. Bioinformatics analysis revealed that TbTim62 contains tetratricopeptide repeat or ankyrin type motifs that would support its ability for protein-protein interactions. However, TbTim62 did not show any homology with the known Tims in other eukaryotes.TbTim62 knockdown, significantly decreased the growth of the procyclic form and inhibited import of nuclear encoded mitochondrial proteins. In addition, depletion TbTim62 also decreases the level of TbTim17 in mitochondria. Crosslinking and pull down analysis confirms the interaction of TbTim62 with TbTim17. So far our data reveals that TbTim62, a trypanosome-specific protein, is associated with TbTim17 and is crtical for mitochondrial protein import in T. brucei. Supported by 2SC1GM081146

224 Structure and Function of Trypanosoma cruzi Flagellar Calcium Binding Protein

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Trypanosoma cruzi flagellar calcium binding protein (FCaBP) is targeted to the lipid raft-rich flagellar plasma membrane via myristate, palmitate, and positively charged lysine residues near the amino terminus. The flagellar localization of FCaBP is further dependent on calcium, a characteristic of mammalian calcium sensor proteins like recoverin (Rv), a calcium sensor in vision. Based on analogy to Rv, we hypothesized that FCaBP undergoes a calcium-dependent change in conformation that results in extrusion or sequestration of the N-terminal acyl groups. This regulates membrane localization and partner protein association. FCaBP does undergo a massive calcium-dependent conformational change that affects its ability to associate with some binding partners. However, an FCaBP binding mutant displays additional calcium-dependent partner associations, indicating that the calcium biochemistry of partner association is not simple. Additional studies including x-ray structure determination indicate: (i) the N-terminal acyl groups of FCaBP are always exposed and able to associate with the flagellar membrane, (ii) FCaBP localizes to the flagellar membrane and associates with lipid rafts regardless of calcium binding and (iii) FCaBP exists as a dimer independent of calcium concentration. A unified model for FCaBP regulation and function that integrates all of the experimental results is presented.

225 Evaluation of safety and immugenicity of live attenuated Leishmania donovani p27 and Centrin gene deleted parasites in hamster model using intradermal route of immunization

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Visceral leishmaniasis is a neglected tropical disease, fatal if untreated. There is no vaccine available against leishmaniasis. Parasite persistence is thought to be important for an effective protective response that may be achieved by immunization with gene-deleted live attenuated parasites that do not cause disease. Recently, we have tested two genetically modified live attenuated parasites, one with a cell division specific centrin1 gene and another with an amastigote specific protein p27 gene deleted. Inoculated by the intravenous route, they were tested for their safety, efficacy, immunogenicity and cross protection against other Leishmania species in animal models. In this study we have repeated the tests of these two vaccine candidates immunizing intradermally to mimic a feasible mode of immunization. We immunized golden hamsters with two different doses (106 and 107) of Ldp27-/- or LdCen-/- parasites via intradermal route. After two months of infection, no parasites were detected in spleen, liver, ear and lymph node by serial dilution suggesting that attenuated parasites are safe and do not persist. Both the attenuated parasites at the 107 dose stimulated strong lymphoproliferative responses, which correlated with higher expression of pro-inflammatory cytokines such as IFN-g, TNF-a and IL-12 resulting in higher iNOS expression in splenocytes, liver and lymph nodes as measured by RT-PCR. This type of immune response generated through intradermal immunization mimics the response through intravenous route which has been demonstrated to be safe and efficacious. Studies are under way to demonstrate whether the immune response generated intradermally by the live attenuated parasites can afford protection against infection through the intradermal route which will mimic natural infection.

226 In vitro screening of compounds identifies RNA editing inhibitors

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RNA editing in kinetoplastid parasites is an essential process for the expression of most mitochondrial proteins. It is carried out by a multi-protein complex, called the editosome. With more than 20 proteins, the editosome is unique to the kinetoplastids and a potential drug target. We previously developed an in vitro fluorescence-based reporter assay to monitor RNA editing. Here, we used this assay to screen a collection of 1280 annotated Library of Pharmacologically Active Compounds at 20 μM concentrations. The screen led to the identification of compounds that showed inhibitory effects on RNA editing. To validate the specificity of inhibition we performed a comprehensive set of in vitro RNA editing assays, including a variation of fluorescence-based reporter assay that bypasses the initial rate-limiting endonucleolytic cleavage step. The characteristics of the alterations indicate which step of the editosome function is affected by the compounds.

227 Computational recognition of cis-regulatory elements in trypanosomatids

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In Trypanosomatids, unlike other eukaryotes, regulation of gene expression occurs mainly at the post-transcriptional level. In this process, cis- and trans-acting elements play important roles in post-transcriptional gene regulation by affecting mRNA maturation, stability and translation rate. Cis-acting elements are usually within the 3´-UTRs, however, systematic identification of these elements are in early stages. Most methods find short conserved sequences or structural patterns based on commonality in a set of related sequences, or conservation across species. In many instances, however, RNA binding proteins (RBPs) (i.e. the trans-acting elements) show preferences for binding to some specific transcripts (bound transcripts) while they do not bind to other transcripts containing the same short sequence motifs (unbound transcripts). In this study, our hypothesis is that special patterns near the conserved motifs facilitate the binding of RBPs. To test this hypothesis, we used a training set of 7 to 100-mers bound and unbound transcripts without considering the nucleotides in each position to fit structural patterns which we then assessed using a held-out test set. We then employed an iterative motif refinement procedure that reduces degeneracy a single base at a time. At each iteration, the motif with the largest mutual information based on its presence at bound transcripts and absence in unbound transcripts was selected. Application of this novel approach to the genome sequence of T. brucei will be presented and show how it can predict gene regulatory networks in this organism.

228 Open chromatin of the VSG active Expression Site is maintained independently of transcription in Trypanosoma brucei

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Trypanosoma brucei is an extracellular parasite that evades the host immune system by periodically changing the cell surface Variant Surface Glycoproteins (VSGs) through antigenic variation. This process relies on transcription by RNA polymerase I of a single VSG gene from one of the 15 subtelomeric Expression Sites (ES). It was previously shown that the chromatin of the active ES is nucleosome-depleted relative to silent ESs. Is transcription necessary for maintaining such open chromatin conformation? Here, we investigated the dynamics of chromatin closure when we induce silencing of the active ES. For this, we adapted a previously published cell-line in which the active ES1 can be inducibly silenced by removing tetracyclin from the medium. Using luciferase as transcriptional reporter, we found that 8 hours after inducing transcription silencing, luciferase activity was reduced to 5%. During this period, the number of cells duplicated and cell death (measured by propidium iodide staining) remained background, indicating that parasites grew normally. FAIRE and Chromatin Immunoprecipitation showed that although ES1 was greatly silenced at 8hr post-induction, its chromatin remained in an open conformation. In contrast, 96 hr post-silencing induction, when parasites have completely switched to a new ES, ES1 is silent and its chromatin is closed. To our knowledge, this is the first description that a nucleosome-depleted genomic region is stably maintained for at least 8hr after a drastic reduction of transcription, suggesting that maintenance of open chromatin at the active ES is transcription-independent. We speculate that the delay between stopping transcription and closing the chromatin is a mechanism used by the parasite during VSG transcriptional switching to select and process transcription of a new ES.

229 TriTrypDB: the functional genomics resource for kinetoplastids

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TriTrypDB (http://tritrypdb.org) is a collaborative endeavor between multiple groups (details on poster). The resource is based on the Eukaryotic Pathogen bioinformatics resource framework (http://eupathdb.org), with funding provided by The Wellcome Trust, The Bill and Melinda Gates Foundation and the National Institutes of Health. TriTrypDB provides an intuitive graphical interface that enables researchers to ask sophisticated in silico questions. Data types in TriTrypDB include the underlying genomic sequences and annotations from over 20 kinetoplastid organisms, transcript level data (SAGE-tags, ESTs, microarray and RNA sequence data including RNAi knockdown), protein expression data, epigenomic data (ChIP-chip and ChIP-seq), and population-level (SNP) and isolate data. In addition, genomic analyses provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (Enzyme commission annotation and GO terms) and evolutionary relationships based on gene orthology. Highlights from TriTrypDB: Community annotation and curation via user comments supported by an annotation team. User comments including images, files, PubMed records, etc can be added to records in TriTrypDB. Comments as well as systematic annotation and curation are incorporated into the genome annotation by dedicated staff based at SBRI and at the University of Liverpool. All updates will be immediately visible through GeneDB and incorporated into TriTrypDB. Graphical search method allows building complex searches in a step-wise manner. Strategies can be saved, modified and shared. An example strategy can be viewed by following this link: http://tritrypdb.org/tritrypdb/im.do?s=2138414b43b897b5 A genomic colocation tool enables searches based on the relative genomic locations. For example, identifying genes based on their location relative to a DNA motif. Column analysis tools are available to generate word cloud graphics and histograms of results. Additional tools will be added in the near future including GO term and pathways enrichment analyses. Upcoming features include metabolomics data integrated via metabolic pathways and host response data.

230 The role of POLIC arginine methylation in Trypanosoma brucei kDNA replication.

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Arginine methylation is a prevalent posttranslational modification to proteins involved in many functions including RNA metabolism, DNA repair, signal transduction, cell cycle checkpoint controls, and protein translocation. The first comprehensive survey of mitochondrial arginine methylproteins was recently completed in Trypanosoma brucei, which identified POLIC as one of several arginine methylated kDNA-associated proteins (Fisk et al, 2013). POLIC and two other essential mitochondrial DNA polymerases (POLIB, POLID) are required for kDNA replication in both procyclic and bloodstream form parasites. Additionally, POLIC dynamically localizes during the cell cycle, with foci accumulating during kDNA S phase and colocalizing with replicating minicircles. We are interested in defining mechanisms that contribute to the regulation of kDNA replication and to the spatiotemporal localization of kDNA-associated proteins. Arginine methylation status may affect these processes. POLIC contains one methylated site near the N-terminus (R420) and two other sites in the polymerase domain (R1250, R1260). To understand the function of POLIC methylation, we prepared procyclic form cells capable of concurrent inducible expression of a POLIC 3'UTR stemloop RNAi trigger and either wild type or the various nonmethylatable (R to K) POLIC variants. UTR-targeted silencing of POLIC resulted in ~80% knockdown of POLIC mRNA and growth inhibition after five days, similar to previously published results. Interestingly, cells displayed asymmetric kDNA division (10.2%), ancillary kDNA (10.2%), and a decrease in cells containing small kDNA (27.7% vs 45%). We are currently investigating how the R420K, R1250K, R1260K mutations affect kDNA replication and POLIC intramitochondrial localization dynamics. These analyses will provide insight into the emerging function of arginine methylation in kDNA-related processes.

231 In silico Targeting of Trypanosomal RNA Editing Ligase 1 for Drug Discovery

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Trypanosoma brucei is the causal agent of Human African trypanosomiasis (HAT). Current HAT drugs show high toxicity, restricted efficacy and increased parasite resistance. A prime target for HAT drug discovery and development is the Trypanosomal RNA editing ligase 1 (TbREL1). Using in silico methods, a number of naphthalene-based inhibitors have been identified that can inhibit the adenylylation step catalyzed by TbREL1 with micromolar range by competing with ATP for the active site. However, we have suggested that although these compounds are able to inhibit adenylylation of TbREL1, this might not be the mechanism through which the drugs exert their effect in the functional biological context. Here, we present alternative in silico methods to identify novel inhibitors against this catalytic step. An extended library of 5.6M drug-like ZINC compounds was docked onto the N-terminal catalytic domain crystal structure of TbREL1 (PDB code 1XDN) via the WILMA-SIE pipeline permitting flexible ligand docking onto a rigid protein structure using an exhaustive sampling approach. Top 825 compounds were narrowed-down to 182 candidates using a consensus scoring scheme relying on SIE, Surflex-Dock and CSCORE algorithms to increase feasibility of hits. Hierarchical clustering of these compounds into 38 chemical classes enabled us to select 65 candidates with varying chemistries which showed good interaction/minimal conflict with catalytic pocket hotspots. All hits selected have a predicted binding affinity of < -8.0kcal/mol. Protein/compound interaction analysis revealed that all 65 candidates interact with residue hotspots PHE209 and VAL286. Other common contacts tabulated involved residues ILE61, LYS87, ARG111 and ARG288. The candidates selected through this study are currently being tested experimentally for inhibitory effect against TbREL1 by our laboratory.

232 Insertion of the Leishmania donovani peroxin-5 into glycosomal membranes.

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Leishmania is the causative agent of a spectrum of devastating diseases, collectively termed leishmaniasis. Leishmania donovani parasites contain a novel organelle called a glycosome which compartmentalizes multiple metabolic and biosynthetic pathways. Correct targeting of this enzymatic machinery to the glycosome is essential for parasite viability. Proteins destined for glycosomal import contain either a C-terminal tripeptide PTS1 (peroxisomal targeting signal 1) or an N-terminal nonapeptide PTS2 (peroxisomal targeting signal 2) signal sequence. The PTS1 and PTS2 sequences are rapidly bound by the Leishmania trafficking receptors peroxin-5 (LdPEX5) and peroxin-7 (LdPEX7), respectively. Glycosomal import is initiated by docking of the cargo loaded receptors to peroxin 14 (LdPEX14), a peripheral membrane protein anchored to the cytosolic face of the glycosomal membrane, a process that mediates the translocation of the cargo proteins into the glycosomal lumen by a yet undefined mechanism. We have demonstrated that an interaction between LdPEX5 and LdPEX14 causes LdPEX5 to undergo a biophysical change from a soluble to an integral membrane protein that is resistant to alkaline carbonate extraction. To this end, in vitro studies are underway to identify the mechanism and domain(s) of LdPEX5 required for membrane insertion using both full length and truncated LdPEX5 in liposome membranes containing LdPEX14. To further characterize LdPEX5-LdPEX14 formed in the lipid bilayer we are using recombinant LdPEX5 and LdPEX14 labelled with the fluorescent dyes Oregon green and Texas Red, respectively. Preliminary results indicate that a domain situated in the N-terminal region of LdPEX5 is involved in mediating the LdPEX5-membrane interaction. Further studies will examine the role of this LdPEX5 domain in translocation of PTS1 proteins into the lumen of L. donovani glycosomes.

233 Descriptive and comparative analysis of the global transcriptome response of Trypanosoma cruzi to different medium conditions

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During its life cycle, Trypanosoma cruzi has to face distinct environments, characterized by a dramatic shift in ambient conditions, as temperature, pH, nutrient disponibility and oxidative bursts, among others. These changes have to be coupled by the parasite, so a specific response evolved to these challenges. Understanding them is very important to identify the mechanisms underlying these processes, as specific modules of co-regulation have evolved. We have transferred T. cruzi Dm28c epimastigotes in exponential growth to distinct media, evaluating nutrient disponibility (TAU, TAU3AAG, PBS), temperature (10oC, 16oC, 37oC, 41oC), pH (4.0, 5.8, 8.5) and oxidative (20µM H2O2, 200µM H2O2), and samples in triplicate were taken from 0, 1, 2, 4, 6 and 24 hours. Total mRNA was extracted and sequenced in a SOLiD4 equipment. We have generated ~3 billion reads, comprising 216 samples and mapped them to the CL Brener genome; differential expression was accessed using the edgeR software, from the Bioconductor project. The amount of differentially expressed genes (DEG) at 10% FDR ranged from ~300 to ~1,500, depending on the evaluated medium. The distinct classes of DEG were mainly constituents of metabolic pathways; also, we have observed different response of protein classes usually considered as regulated by stress (heat shock proteins, for instance). More interestingly, comparing the different transcriptome responses against each other, we were able to identify a general modulated core, but most of the genes were modulated in specific responses. Taken together, these results represent the first global transcriptomics analysis of T. cruzi response to several environment changes; these data is integrated in our database of gene expression regulation and being used for identification of co-regulation modules.

234 TrypanoGEN: an integrated approach to the identification of genetic determinants of susceptibility to trypanosomiasis

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The TryapanoGEN network was recently funded by the Wellcome Trust to develop an integrated approach to the study of human susceptibility to African trypanosomiasis. The network (all partners listed on the poster) consists of an interdisciplinary research team incorporating parasitologists, geneticists, genome analysts, clinicians, ethicists and bioinformaticians across East and West Africa. The main objectives of the network are: (1) To create an extensive biobank of both retrospective and prospective samples with extensive clinical phenotyping, (2) To generate a database of human genetic variation from different African countries that will be available to the wider scientific community (3) To identify genes involved in human susceptibility to African Trypanosomiasis. Underpinning these scientific objectives is the focus on enhancing local research capacity via development of training in diagnosis, sampling, biobanking and genomic-scale technologies. Importantly, this project will contribute to African research in general by (i), allowing for data mining of informative SNPs, (ii) improving accuracy of implementation strategies used in genome-wide association studies, providing new African genome reference panels and (iii) training future African research leaders. The network will also integrate with the other projects funded under the Human Heredity and Health in Africa initiative (http://h3africa.org) as well as look to expand the network to include studies on other vector-borne parasitic diseases.

235 A proteomics analysis of the trypanosome transition zone

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The trypanosome flagellum is a multifunctional organelle involved in motility, environmental sensing and adherence in various different lifecycle stages. The flagellum transition zone, positioned between the distal end of the basal bodies and the proximal end of the 9+2 axoneme, is becoming increasingly recognized as of central importance to flagellum function. The transition zone maintains the different compositions of the flagellum and cell body by regulating traffic to and from the flagellum and by providing a diffusion barrier at the base of the flagellum. Moreover, many of the “ciliopathies” such as Meckel and Joubert syndrome are now being recognized as transition zone diseases. Despite this, in trypanosomes few proteins have been identified that localize to this area and the proteins that make up the characteristic structures of the transition zone have not been identified in any organism. To address this, I have used immune-precipitation and mass spectrometry to purify the transition zone and identify its protein components. Candidate proteins were genetically tagged with a green fluorescent protein to verify their localization to the transition zone. To investigate the functions of these transition zone proteins, I have generated reporter cell lines that will allow me to determine their role in maintaining the different environments of the flagellum and cell body.

236 The role of an HMG box-containing proteins, TbKAP4, in Trypanosoma brucei kDNA

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T. brucei kinetoplast DNA (kDNA) is a massive, highly condensed DNA network of interlocked minicircles and maxicircles. It remains unclear how this complex network is organized and rearranged throughout the cell cycle. In a search for kDNA architectural components, we examined a family of small, basic proteins related to the kDNA-associated proteins (KAP) (Xu C. W., Hines, Engel, and Ray D. S., 1996). Two of these proteins, TbKAP4.1 and TbKAP4.2, are almost identical and differ by only one amino acid residue. They are HMGB1-like proteins containing two degenerate HMG boxes. The HMG box is a versatile DNA- and protein- binding domain, by which HMG box (HMGB) proteins play essential roles in recognition and maintenance of DNA, chromatin remodeling, and genome architecture. Immunofluorescence studies showed TbKAP4 localizes throughout kinetoplast and predominantly binds to newly replicated minicircles. Knockdown of TbKAP4 leads to cell death, kDNA disorganization and segregation defects. Few cells lose kDNA during a 6-day RNAi experiment. Southern blots show total minicircle level only slightly changed. These data suggest TbKAP4 plays a role in kDNA organization and a much smaller role in kDNA replication. TbKAP4.2 overexpression causes kDNA disorganization and interestingly produces extra mini-kinetoplasts (Miyahira & Dvorak, 1994) in > 50% of cells, which unexpectedly position at the anterior part of the cell. Electron microscopy and FISH confirmed these extra mini-kDNAs are inside mitochondrial matrix and contain minicircles. While the nature of the ‘mini-kDNA’ is unclear, our results raise the possibility that TbKAP4 forms part of a scaffold upon which kDNA is organized and condensed, through its HMG-box-mediated DNA- and protein- binding abilities. Studies to test these hypotheses are underway.

237 Characterization of a novel Leishmania aminopeptidase implicated in parasite virulence

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Protozoan parasites of the genus Leishmania are responsible for important neglected diseases in humans and animals, ranging from self-healing cutaneous lesions to fatal visceral manifestations. During the infectious cycle, Leishmania is exposed to various environmental stress signals in insect and mammalian hosts, which trigger parasite development into the virulent metacyclic and pathogenic amastigote life cycle stages. Extracellular signals are translated into stage-specific gene expression by reversible protein phosphorylation that is controlled by a network of protein kinases and phosphatases. Despite the importance of environmental sensing in transmission and intracellular infection, our understanding on mechanisms underlying these processes remains very poor. Protein phosphorylation is an important process in Leishmania differentiation and stage-specific mitogen-activated protein kinases (MAPKs) are likely to play a crucial regulatory role in stage-specific gene expression and parasite virulence. 2D-DIGE quantitative phospho-proteomics analysis of LmaMPK7 transgenic parasites correlated increased LmaMPK7 activity with statistically significant increased phosphorylation of a putative aminopeptidase (AP1). Bioinformatic analysis revealed similarities to other aminopeptidase-like proteins in higher eukaryotes. Attempts to generate a null-mutant were unsuccessful, suggesting an essential function of this protein in the biology of the parasite. A gain-of-function approach allowed us to gain insight into the function of this novel gene. Transgenic parasites over-expressing AP1 showed growth defects through all stages of the life cycle. This growth delay did not impair their ability to go under metacyclogenesis. In vitro and in vivo infection studies showed attenuation in virulence. Comparative proteomics of AP1 transgenic parasites suggested a direct role in translation. Downstream targets of MAPKs are largely uncharacterized and this work underscores its potential as novel drug targets.

238 Control of Protein Expression in Trypanosoma brucei

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Contrasting physiological requirements for T. brucei survival between procyclic (vector) and bloodstream (mammal) forms necessitate different molecular processes and therefore changes in protein expression. Transcriptional regulation is particularly interesting in T. brucei because the arrangement of genes is polycistronic; however genes which are transcribed together are cleaved into separate mRNAs by trans-splicing and are individually regulated. While multiple stage-specific transcripts have been identified, studies using RNA-seq and microarrays found marginal changes in overall transcript levels suggesting that T. brucei predominantly regulate gene expression by post-transcriptional mechanisms. Our working hypothesis is that in addition to mRNA stability, mRNA recruitment to ribosomes may play a significant role in the regulation of gene expression in T. brucei. To approach this question, we performed RNA-seq of total, subpolysomal, and polyribosomal polyA+ mRNA purified from bloodstream form and procyclic form T. brucei followed by bioinformatic analysis comparing transcript profiles. Complementary to transcriptomic profiling, gene regulation in trypanosomes will also be examined in the context of the 7-methylguanosine cap attached to the 5’ end of the first transcribed nucleotide. Interestingly, in addition to the capping enzymes identified in other eukaryotes, trypanosomatids have an additional guanylyltransferase and methyltransferase in the form of a bifunctional enzyme. The purpose of the bifunctional capping enzyme remains unclear, so we propose to study the structure and essentiality of this enzyme in bloodstream form T. brucei and investigate its therapeutic potential.

239 The roles of 3’-exoribonucleases and the exosome in trypanosome mRNA degradation

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The degradation of eukaryotic mRNAs can be initiated by deadenylation, decapping or endonuclease cleavage. This is followed by 5’-3’ degradation by homologues of Xrn1, and/or 3’-5’ degradation by the exosome. We previously reported that in African trypanosome Trypanosoma brucei, deadenylation is the bottleneck for degradation of most mRNAs, and that depletion of the major 5’-3’ exoribonuclease XRNA preferentially stabilises unstable mRNAs. We now show that depletion of either CAF1 and CNOT10, two components of the principal deadenylation complex, strongly inhibits degradation of most mRNAs. RNAi targeting another deadenylase, PAN2, or RRP45, a core component of the exosome, preferentially stabilised mRNAs with intermediate half-lives. RRP45 depletion resulted in a 5’ bias of mRNA sequences, suggesting action by a distributive 3’-5’ exoribonuclease. Results suggested that the exosome is involved in the processing of trypanosome snoRNAs. There was no correlation between effects on half-lives and on mRNA abundance.

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