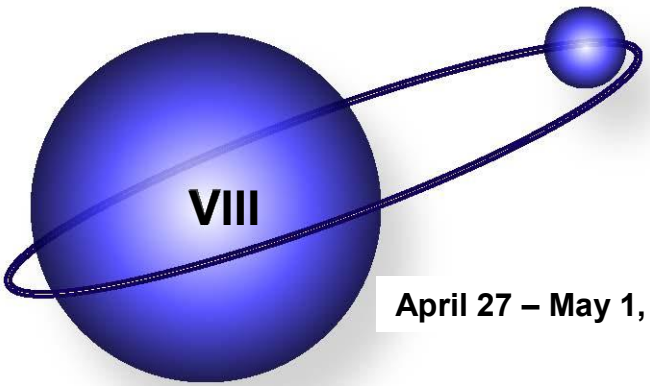
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory · Woods Hole · MA · USA



April 27 – May 1, 2019

Eight Kinetoplastid Molecular Cell Biology Meeting

April 27 – May 1, 2019

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

The meeting was founded by George A.M. Cross in 2005

Organizers

George A.M. Cross	**2005-2011**
Christian Tschudi	**2013-2019**

KMCBM 2019 Acknowledgements

The organizer wishes to thank:

The Program Committee

Barbara Burleigh (Harvard T. H. Chan School of Public Health, Boston, USA)
James D. Bangs (University at Buffalo, Buffalo, USA)
Stephen M. Beverley (Washington University School of Medicine, St. Louis, USA)
Keith R. Matthews (The University of Edinburgh, Edinburgh, Scotland, UK)

The Staff at MBL:

Paul Anderson and the MBL Housing and Conference Staff for registration and housing; All the IT AV Support staff and the staff in Sodexo Food Service at the MBL.

Cover Design: Markus Engstler

KMCBM 2019 Program

Saturday, April 27

02:00 – 05:00	Arrival, Registration and Poster Session A setup
04:00 – 06:30	Greeting and Dinner
07:00 – 09:00	Session I: VSG (chair: Mark Carrington)
09:00 – 11:00	Mixer

Sunday, April 28

07:00 – 08:30	Breakfast
08:45 – 11:45	Session II: Biochemistry/Metabolism (chair: Ken Stuart)
12:00 – 01:30	Lunch
02:00 – 04:30	Session III: Cell Biology (chair: Kimberly Paul)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session A
09:00 – 11:00	Mixer & Poster A/B Changeover

Monday, April 29

07:00 – 08:30	Breakfast
08:45 – 11:45	Session IV: Pathogenesis I (chair: Luisa Figueiredo)
12:00 – 01:30	Lunch
01:30 – 06:00	Free Time
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session B
09:00 – 11:00	Mixer & Poster B/C Changeover

Tuesday, April 30

07:00 – 08:30	Breakfast
08:45 – 11:45	Session V: Cell Cycle/Cytokenisis (chair: Michele Klingbeil)
12:00 – 01:30	Lunch
02:00 – 04:30	Session VI: Gene Expression (chair: Ruslan Afasizhev)
06:00 – 07:00	Dinner
07:00 – 09:00	POSTER PRESENTATIONS: Session C
09:00 – 11:00	Mixer

Wednesday, May 1

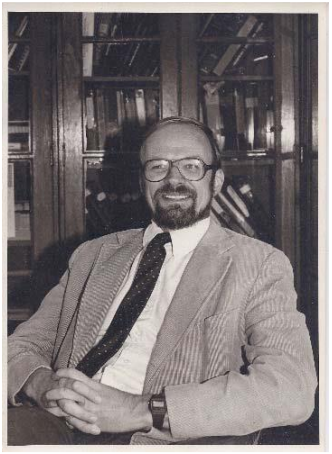
07:00 – 08:30	Breakfast
08:45 – 11:15	Session VII: Pathogenesis II (chair: Megan Povelones)
12:00 – 01:30	Lunch or lunch boxes

ROOMS MUST BE VACATED BY **10:00 AM** ON WEDNESDAY

In Memoriam:

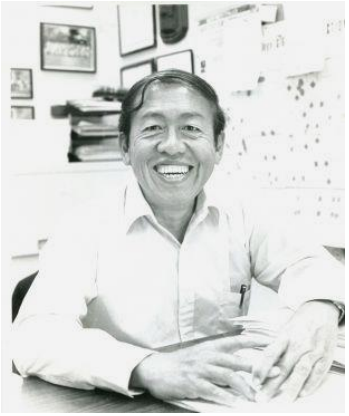
Paul T. Englund, Ph.D.

1938-2019



C.C. Wang, Ph.D.

1936-2017



SESSION I:
VSG
Mark Carrington, Chair.
Saturday 7:00 pm
04/27/19

1A Identification of novel ESB associated proteins in bloodstream form Trypanosoma brucei
Budzak, James Jones, Robert (Sir Alexander Fleming Building, Dept. of Life Sciences, Imperial College London, U.K.); Tschudi, Christian; Kolev, Nikolay G. (Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT 06536, USA.); Rudenko, Gloria (Sir Alexander Fleming Building, Dept. of Life Sciences, Imperial College London, U.K.)

1B A chromatin-associated VEX/CAF-1 complex drives VSG single gene choice and epigenetic inheritance in Trypanosoma brucei
Faria, Joana Glover, Lucy; Hutchinson, Sebastian (Institut Pasteur Paris); Boehm, Cordula; C. Field, Mark; Horn, David (Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee)

1C m6A methylation of the polyA-tail regulates VSG transcript stability in trypanosomes
Viegas, Idalio Rodrigues, João; Macedo, Juan (Instituto de Medicina Molecular); Aresta Branco, Francisco (Division of Immune Diversity, German Cancer Research Center, Heidelberg, Germany.); Jaffrey, Samie (Department of Pharmacology, Weill Cornell Medicine, Cornell University); Figueiredo, Luisa (Instituto de Medicina Molecular)

1D Trypanosoma brucei RAP1 has an RNA binding activity that is essential for VSG monoallelic expression
Gaurav, Amit K. Afrin, Marjia (Cleveland State University); Yang, Xian; Pan, Xuehua (The Hong Kong Polytechnic University); Sandhu, Ranjodh (University of California, Davis); Zhao, Yanxiang (The Hong Kong Polytechnic University); Li, Bibo (Cleveland State University)

1E Active expression site ESAGs are not essential in vitro, but their presence alters VSG switching
Chamberlain, James Gadelha, Catarina (University of Nottingham, Nottingham, United Kingdom)

1F Developmental competence and antigen switch frequency can be uncoupled in Trypanosoma brucei
McWilliam, Kirsty Mugnier, Monica (Johns Hopkins University); Morrison, Liam; Matthews, Keith (University of Edinburgh)

TT1G TURNOVER OF VSG IN TRYPANOSOMA BRUCEI
Garrison, Paige Umaer, Khan; Bush, Peter; Bangs, Jay (University at Buffalo)

TT1H How DNA breaks contribute to mosaic VSG formation in Trypanosoma brucei?
Romero-Meza, Gabriela Mugnier, Monica (Johns Hopkins Bloomberg School of Public Health)

SESSION II: Biochemistry/Metabolism
Ken Stuart, Chair.
Sunday 8:45 am
04/28/19

2A Glycerol, a new key player in the central carbon metabolism of African trypanosomes
Bringaud, Frédéric MFP (CNRS UMR-5234), University of Bordeaux, Bordeaux, France

2B The Trypanosoma UDP-glucose pyrophosphorylase is active in the cytosol and glycosomes and is imported into the organelle by piggybacking
Villafraz, Oriana; Baudouin, Hélène; Mazet, Muriel (MFP (CNRS UMR-5234), University of Bordeaux, Bordeaux, France); Kulyk, Hanna; Portais, Jean-Charles (LISBP (CNRS, INRA, INSA), University of Toulouse, Toulouse, France)

2C Responses to reduced glucose transport in Trypanosoma brucei: gluconeogenesis and signaling
Kovarova, Julie Hutchinson, Sebastian; Horn, David (University of Dundee)

2D Trypanosoma cruzi intracellular amastigote glutamine metabolism and its impact on susceptibility to ergosterol biosynthesis inhibitors
Dumoulin, Peter Burleigh, Barbara (Harvard T.H. Chan School of Public Health)

2E A foot in the door: Understanding nutrient stress tolerance and adaptive gene regulation in Leishmania donovani through the lens of a purine-responsive transporter
Licon, Haley Soysa, Radika (University of Washington); Landfear, Scott; Yates, Phillip (Oregon Health and Science University)

2F Remodeling the mitochondrial proteome and metabolic networks of Trypanosoma brucei during in vitro insect stage differentiation
Zikova, Alena Dolezelova, Eva (Biology Centre); Levin, Michal; Dejung, Mario (Institute of Molecular Biology); Kunzova, Michaela; Panicucci, Brian (Biology Centre); Janzen, Christian; Butter, Falk (University Wuerzburg)

2G Deciphering the mitochondrial quality control mechanism in Trypanosoma brucei
Dewar, Caroline Oeljeklaus, Silke; Suppanz, Ida; Peikert, Christian (University of Freiburg); Mani, Jan (University of Bern); Warscheid, Bettina (University of Freiburg); Schneider, André (University of Bern)

TT2H Targeting the ubiquitin system of Leishmania Mexicana
Burge, Rebecca Damianou, Andreas; Catta-Preta, Carolina (The University of York); Rodenko, Boris (UbiQ); Mottram, Jeremy C. (The University of York)

TT2I Identifying highly divergent glycosyltransferases in the African Trypanosome
Duncan, Samuel Damerow, Manuela; Ferguson, Michael (The University of Dundee)

SESSION III:
Cell Biology
Kimberly Paul
Sunday 2:00 pm
04/28/19

3A Cryo electron tomography with Volta phase plate reveals novel structural foundations of axoneme motility in the pathogen Trypanosoma brucei

Imhof, Simon Zhang, Jiayan (Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California, USA); Wang, Hui (California NanoSystems Institute, UCLA); Huy Bui, Khanh (Department of Anatomy and Cell Biology, McGill University, Montreal, CA); Nguyen, Hoangkim (Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California, USA); Atanosov, Ivo; Hui, Wong; Zhou, Hong (California NanoSystems Institute, UCLA); Hill, Kent (Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California, USA)

3B The Leishmania flagellum attachment zone is critical for flagellar pocket shape and development in the sand fly and pathogenicity in the host

Sunter, Jack Yanase, Ryuji (University of Hyogo); Wang, Ziyin (University of Oxford); Catta-Preta, Carolina (University of York); Moreira-Leite, Flavia (University of Oxford); Myskova, Jitka; Pruzinova, Katerina; Volf, Petr (Charles University); Mottram, Jeremy (University of York); Gull, Keith (University of Oxford)

3C Structural studies of the interaction between FPC3 and FPC4, two components of the flagellar pocket collar in Trypanosoma brucei

Pivovarova, Yulia Majneri, Paul; Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria); Robinson, Derrick R.; Bonhivers, Melanie (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)

3D Characterization of TAP110, a mitochondrial protein associated with the TAC, in Trypanosoma brucei

Amodeo, Simona Schimanski, Bernd; Ochsenreiter, Torsten (University of Bern, Switzerland)

3E Processing and targeting of cathepsin L (TbCatL) to the lysosome in Trypanosoma brucei

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

TT3F Swim like your lifecycle depends on it: Investigating chemotaxis and navigation in Leishmania parasites

Walrad, Pegine B. Findlay, Rachel; Osman, Mohamed (York Biomedical Research Institute); Gadelha, Hermes (Department of Mathematics); Kaye, Paul (York Biomedical Research Institute); Rogers, Matthew (London School of Hygeine and Tropical Medicine); Wilson, Laurence (Biological Physical Sciences Institute, University of York)

TT3G The dual FAZ domain-localizing protein FAZ27 cooperates with FLAM3 and ClpGM6 to control morphology transitions in Trypanosoma brucei

AN, TAI ZHOU, QING; HU, HUIQING; LI, ZIYIN (University of Texas Medical School)

TT3H TrypTag: Biological lessons and opportunities from a molecular atlas of trypanosomes

Dean, Samuel Billingdon, Karen (University of Oxford); Halliday, Clare (Oxford Brookes); Madden, Ross (University of Oxford); Carrington, Mark (University of Cambridge); Hertz-Fowler, Christiane (University of Liverpool); Vaughan, Sue (Oxford Brookes); Gull, Keith (University of Oxford); Sunter, Jack (Oxford Brookes); Wheeler, Richard (University of Oxford)

TT3I High frame rate multi-colour and multi-focal plane microscopy to gain new insights into trypanosomatid parasite swimming

Wheeler, Richard J. (University of Oxford)

TT3K UNRAVELLING THE ROLE OF PROTEIN KINASES IN LEISHMANIA DIFFERENTIATION

Baker, Nicola Catta-Preta, Carolina; Neish, Rachel; Powell, Ben; Ferreira, Eliza; Geoghegan, Vincent; Newling, Katherine; Pitchford, John; Mottram, Jeremy (University of York)

SESSION IV:
Pathogenesis I
Luisa Figueiredo, Chair.
Monday 8:45 am
04/29/19

4A The life of trypanosomes in the tsetse fly
Roditi, Isabel (Institute of Cell Biology, Bern, Switzerland)

4B Chemotaxis in trypanosomes – it's all about the basics
Shaw, Sebastian Naguleswaran, Arunasalam; Roditi, Isabel (Institute of Cell Biology, Bern, Switzerland)

4C A bar-seq fitness screen of Leishmania CRISPR-Cas9 knockout mutants shows the importance of motility in colonization of sandflies
Beneke, Tom Smith, James (University of Oxford, Sir William Dunn School of Pathology); Hookway, Edward (Research Department of Pathology, University College London); Becvar, Tomas; Myskova, Jitka; Lestinova, Tereza; Sadlova, Jovana; Volf, Petr (Department of Parasitology, Faculty of Science, Charles University); Wheeler, Richard (Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford); Gluenz, Eva (University of Oxford, Sir William Dunn School of Pathology)

4D Dramatic changes in gene expression in different forms of Crithidia fasciculata reveal potential mechanisms for insect-specific adhesion in kinetoplastid parasites
Povelones, Megan L. Filosa, John (University of Pennsylvania); Berry, Corbett (Drexel University); Ruthel, Gordon (University of Pennsylvania); Dudkin, Elizabeth (Penn State Brandywine); Povelones, Michael (University of Pennsylvania)

4E The generation of advanced human skin tissue models allows the controlled investigation of developmental processes of tsetse-transmitted African trypanosomes in the dermis
Reuter, Christian Finger, Tamara; Fey, Philipp; Krüger, Timothy; Walles, Heike; Groeber-Becker, Florian; Engstler, Markus

4F A Trypanosoma brucei factor H receptor and its mechanism of interaction with the mammalian complement system
Macleod, Olivia JS Bart, Jean-Mathieu (UMR INTERTRYP-CIRAD); MacGregor, Paula (University of Cambridge); Peacock, Lori (University of Bristol); Hester, Svenja (University of Oxford); Rust, Steven (MedImmune); Ravel, Sophie (UMR INTERTRYP-CIRAD); Trevor, Camilla (University of Cambridge); Winter, Ralph (MedImmune); Mohammed, Shabaz (University of Oxford); Gibson, Wendy (University of Bristol); Vaughan, Tristan J (MedImmune); Taylor, Martin C (London School of Hygiene and Tropical Medicine); Higgins, Matthew K (University of Oxford); Carrington, Mark (University of Cambridge)

4G Baboon apolipoprotein L-1 forms pH-independent membrane channels, thus conferring immunity to the human pathogen T. b. gambiense
Verdi, Joey Thomson, Russell; Raper, Jayne (Hunter College CUNY)

4H Structure of the trypanosome transferrin receptor reveals mechanisms of ligand recognition and immune evasion
Trevor, Camilla Gonzalez-Munoz, Andrea (MedImmune); Macleod, Olivia (University of Cambridge); Woodcock, Peter (University of Oxford); Rust, Steven; Vaughan, Tristan (MedImmune); Garman, Elspeth (University of Oxford); Minter, Ralph (MedImmune); Carrington, Mark (University of Cambridge); Higgins, Matthew (University of Oxford)

SESSION V:
Cell Cycle/Cytokenisis
Michele Klingbeil, Chair.
Tuesday 8:45 am
04/30/19

5A Protein kinases regulating kinetochore function in Trypanosoma brucei
Mottram, Jeremy; Saldivia, Manuel (University of York); Rao, Srinivasa (Novartis Institute for Tropical Diseases)

5B Analysis of the interface between microtubules and unconventional kinetochores
Hayashi, Hanako Llauró, Aida; Bailey, Megan E. (Department of Physiology and Biophysics, University of Washington); Wilson, Alex; Ludzia, Patryk (Department of Biochemistry, University of Oxford); Höög, Johanna (Department of Chemistry and Molecular Biology, University of Göthenburg); Asbury, Charles L. (Department of Physiology and Biophysics, University of Washington); Akiyoshi, Bungo (Department of Biochemistry, University of Oxford)

5C Organising the cell cycle in the absence transcriptional control: Dynamic phosphorylation co-ordinates the Trypanosoma brucei cell cycle post-transcriptionally
Urbaniak, Michael D. Benz, Corinna (Lancaster University)

5D Nucleopores complexes are essential in the maintenance of ploidy in Trypanosomatid parasites.
Sterkers, Yvon Yagoubat, Akila; Stanojcic, Slavica (University Montpellier); Berry, Laurence; Crobu, Lucien (CNRS); Kuk, Nada; Morelle, Christelle; Lachaud, Laurence; Bastien, Patrick (University Montpellier)

5E Cell cycle checkpoint proteins HUS1 and RAD9 differentially modulate gene amplification and genome variability in Leishmania
Obonaga, Ricardo Damasceno, Jeziel Dener (University of Glasgow); L. Reis-Cunha, João (Universidade Federal de Minas Gerais(UFMG)); V. Santos, Elaine (University of São Paulo(FMRP-USP)); L. A. Silva, Gabriel; Virgilio, Stela (University of São Paulo (FMRP-USP)); S. Bastos, Matheus (University of São Paulo); C. Bartholomeu, Daniella (Universidade Federal de Minas Gerais (UFMG)); McCulloch, Richard (University of Glasgow); Tosi, Luiz Ricardo Orsini (University of São Paulo (FMRP-USP))

5F Robust loss-of-fitness measurements, combinatorial RNAi and genome-wide epistatic interactions by Direct RNAi Fragment-sequencing (DRiF-Seq)
Wickstead, Bill Brusini, Lorenzo; D'Archivio, Simon; Stretton, Owen; Gadelha, Catarina (University of Nottingham)

5G Long-term Trypanosoma brucei live-cell imaging using agarose micro-chambers identifies novel RNAi phenotypes and protein localization during cell division
de Graffenried, Christopher L. Muniz, Richard; Sladewski, Thomas (Brown University); Renner, Lars (Leibniz Institute of Polymer Research); Perry, Jenna (Brown University)

TT5H Characterising Leishmania kinetochores with XL-BioID
Geoghegan, Vincent Jones, Nathaniel; Dowle, Adam; Larson, Tony; Mottram, Jeremy (University of York)

TT5I Identifying novel factors associated with trypanosome DNA replication forks using nascent DNA proteomics

Rocha-Granados, Maria Bermudez, Yahaira; Dodard, Garvin (University of Massachusetts); Gunzl, Arthur (UConn Health); Klingbeil, Michele M. (University of Massachusetts, Amherst, Amherst, MA)

SESSION VI:
Gene Expression
Ruslan Afasizhev, Chair.
Tuesday 2:00 pm
04/30/19

6A A Novel PNUTS-PP1 Phosphatase Complex Orchestrates RNA Pol II Transcription Termination in T. brucei

Kieft, Rudo Zhang, Yang (Department of Biochemistry and Molecular Biology, University of Georgia); Marand, Alexandre P. (Department of Genetics, University of Georgia); Moran, Jose Dagoberto (Department of Biochemistry and Molecular Biology, University of Georgia); Bridger, Robert; Wells, Lance (Complex Carbohydrate Research Center, University of Georgia); Schmitz, Robert J. (Department of Genetics, University of Georgia); Sabatini, Robert (Department of Biochemistry and Molecular Biology, University of Georgia)

6B Genome-wide mapping reveals R-loop activity in genome maintenance at sites of pre-mRNA processing in Leishmania.

Damasceno, Jeziel Dener Reis-Cunha, João L (Universidade Federal de Minas Gerais(UFMG)); Briggs, Emma; Lapsley, Craig; Crouch, Kathryn (University of Glasgow); Bartholomeu, Daniella C. (Universidade Federal de Minas Gerais(UFMG)); McCulloch, Richard (University of Glasgow)

6C EpiRNomics in trypanosomatids: impact on parasite cycling between the mammalian and insect host

Michaeli, Shulamit Rajan, K. Shanmugha (Bar-Ilan University); Chikne, Vaibhav (Bar-Ilan Unversity); Cohen Chalamish, Smadar (Bar-Ilan university); Doniger, Tirza (Bar-Ilan University); Sirvastava, Ankita (University of Connecticut Health Center); Unger, Ron (Bar-Ilan University); Gunzl, Arthur (University of Connecticut Health Center); Tschudi, Christian (Yale University)

6D The extended family of retrotransposon hotspot genes in trypanosomes: junk DNA or doing things differently?

Florini, Francesca Naguleswaran, Arunasalam (IZB, University of Bern, Switzerland); Gharib, Walid H. (Interfaculty Bioinformatics Unit, University of Bern, Switzerland); Bringaud, Frédéric (Laboratoire de Microbiologie Fondamentale et Pathogénicité (MFP), Université de Bordeaux, France); Roditi, Isabel (IZB, University of Bern, Switzerland)

6E Iron-dependent control of transferrin receptor expression in Trypanosoma brucei

Tiengwe, Calvin J Cornell, Lucy; Gilabert-Carbajo, Carla; Lai, Zhihao (Imperial College London); D. Bangs, James (University at Buffalo)

6F Trypanosome brucei RNase H2 is an essential nuclear enzyme with roles in both transcription initiation and antigenic variation.

Briggs, Emma Crouch, Kathryn; Lemgruber, Leandro (WCIP, University of Glasgow); Hamilton, Graham (Glasgow Polyomics); Lapsley, Craig; McCulloch, Richard (WCIP, University of Glasgow)

6G Histone variants link genome architecture and antigenic variation in Trypanosoma brucei

Cosentino, Raul O Müller, Laura SM; Brink, Benedikt; Siegel, T. Nicolai (Department of Veterinary Sciences, Experimental Parasitology. Ludwig-Maximilians-Universität München)

TT6H An ORF-based whole-genome gain-of-function library for Trypanosoma brucei

Quinn, McKenzie Gomez, Stephanie (The George Washington University); Schultz, Danae (Harvey Mudd College); Kim, Hee-Sook (Cleveland State University); Hovel-Miner, Galdriel (The George Washington University)

TT6I UTR-seq: hundreds of new regulatory 3'-untranslated regions in African trypanosomes

Trenaman, Anna Wall, Richard; Horn, David (University of Dundee)

TT6K Characterization of putative chromo- and SET-domain transcription regulators in Trypanosoma brucei

Staneva, Desislava Carloni, Roberta; Auchynnikava, Tatsiana; Tong, Pin; Matthews, Keith; Allshire, Robin (The University of Edinburgh)

TT6L Intriguing roles assigned to Leishmania eIF4E paralogs by structure-function studies and CRISPR-Cas 9-mediated knockout approach

Shapira, Michal Tupperwar, Nitin; Shrivastava, Rohit; Kamus-Elimeleh, Dikla; Orr, Irit (Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel); Meleppattu, Shimi; Leger-Abraham, Melissa (Microbiology Department, Blavatnik Institute, Harvard Medical School, Boston, MA); Wagner, Gerhard (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA)

SESSION VII:
Pathogenesis II
Megan Povelones, Chair.
Wednesday 8:45 am
05/1/19

7A Leishmania RNA Viruses and Parasite Virulence
Beverley, Stephen M. Akopyants, Natalia; Lye, Lon-Fye (Washington University School of Medicine)

7B Unconventional activation of a DYRK kinase implicated in the Trypanosoma brucei quorum-sensing response
Cayla, Mathieu McDonald, Lindsay (University of Edinburgh); MacGregor, Paula (University of Cambridge); Matthews, Keith R. (University of Edinburgh)

7C Metabolic sensing in African trypanosomes: A possible role of aquaglyceroporins and glycerol along the entire parasite life cycle
CALVO ALVAREZ, ESTEFANIA Pineda, Erika (Centre de Résonance Magnétique des Systèmes Biologiques (CRMSB), Université de Bordeaux); Cren-Travaillé, Christelle (Trypanosome Transmission Group, Trypanosome Cell Biology Unit, Institut Pasteur); Biran, Marc (Centre de Résonance Magnétique des Systèmes Biologiques (CRMSB), Université de Bordeaux); Crouzols, Aline (Trypanosome Transmission Group, Trypanosome Cell Biology Unit, Institut Pasteur); Bastin, Philippe (Trypanosome Cell Biology Unit, Institut Pasteur); Bringaud, Frédéric (Laboratoire de Microbiologie Fondamentale et Pathogénicité, Centre de Résonance Magnétique des Systèmes Biologiques, Université de Bordeaux); Rotureau, Brice (Trypanosome Transmission Group, Trypanosome Cell Biology Unit, Institut Pasteur)

7D Glycerol: a tissue-specific stumpy inducing factor in trypanosomes?
Guegan, Fabien Teixeira, Alexandra; Machado, Henrique (Instituto de Medicina Molecular); Calvo Alvarez, Estefania; Rotureau, Brice (Pasteur Institut); Figueiredo, Luisa (Instituto de Medicina Molecular)

7E A coordinated expression of TbMYND and RBP6 allows development of Trypanosoma brucei infectious forms in the tsetse
Casas-Sanchez, Aitor Lopez-Escobar, Lara; Cansado-Utrilla, Cintia; Haines, Lee (Liverpool School of Tropical Medicine); Darby, Alister (University of Liverpool); Shamsani, Jannah; Walrad, Pegine (University of York); Acosta-Serrano, Alvaro (Liverpool School of Tropical Medicine)

7F Two cold shock domain proteins in Trypanosoma brucei are involved in the developmental regulation of metacyclic VSG genes
Toh, Justin Nkouawa, Agathe; Rojas-Sánchez, Saúl; Shi, Huafang; Lei, Yuling; Kolev, Nikolay G. (Yale School of Public Health); Toh, Justin (Yale School of Public Health, New Haven, CT); Tschudi, Christian (Yale School of Public Health)

7G BCAAs reshape the metabolism and infection capability of Trypanosoma cruzi metacyclic trypomatigotes
de Freitas Nascimento, Janaína da Silva Damasceno, Flávia; Nakamura Rapado, Ludmila; Barison, Maria Julia; Marsiccobetre, Sabrina (Universidade de Sao Paulo); de Luna Vitorino, Francisca Nathália (Instituto Butantan); Merino, Emilio Fernando (Virgina Tech); Cassera, Maria Belen (Virginia Tech); Pinheiro Chagas da Cunha, Julia (Instituto Butantan); Silber, Ariel Mariano (Universidade de Sao Paulo)

POSTERS: Session A
Sunday 7:00 pm
04/28/19

1 - TT1G TURNOVER OF VSG IN TRYPANOSOMA BRUCEI
Garrison, Paige Umaer, Khan; Bush, Peter; Bangs, Jay (University at Buffalo)

2 - TT1H How DNA breaks contribute to mosaic VSG formation in Trypanosoma brucei?
Romero-Meza, Gabriela Mugnier, Monica (Johns Hopkins Bloomberg School of Public Health)

3 - TT2H Targeting the ubiquitin system of Leishmania Mexicana
Burge, Rebecca Damianou, Andreas; Catta-Preta, Carolina (The University of York); Rodenko, Boris (UbiQ); Mottram, Jeremy C. (The University of York)

4 - TT2I Identifying highly divergent glycosyltransferases in the African Trypanosome
Duncan, Samuel Damerow, Manuela; Ferguson, Michael (The University of Dundee)

5 – TT3F Swim like your lifecycle depends on it: Investigating chemotaxis and navigation in Leishmania parasites
Walrad, Pegine B. Findlay, Rachel; Osman, Mohamed (York Biomedical Research Institute); Gadelha, Hermes (Department of Mathematics); Kaye, Paul (York Biomedical Research Institute); Rogers, Matthew (London School of Hygeine and Tropical Medicine); Wilson, Laurence (Biological Physical Sciences Institute, University of York)

6 – TT3G The dual FAZ domain-localizing protein FAZ27 cooperates with FLAM3 and ClpGM6 to control morphology transitions in Trypanosoma brucei
AN, TAI ZHOU, QING; HU, HUIQING; LI, ZIYIN (University of Texas Medical School at Houston)

7 - TT3H TrypTag: Biological lessons and opportunities from a molecular atlas of trypanosomes
Dean, Samuel Billingdon, Karen (University of Oxford); Halliday, Clare (Oxford Brookes); Madden, Ross (University of Oxford); Carrington, Mark (University of Cambridge); Hertz-Fowler, Christiane (University of Liverpool); Vaughan, Sue (Oxford Brookes); Gull, Keith (University of Oxford); Sunter, Jack (Oxford Brookes); Wheeler, Richard (University of Oxford)

8 - TT3I High frame rate multi-colour and multi-focal plane microscopy to gain new insights into trypanosomatid parasite swimming
Wheeler, Richard J.(University of Oxford)

9 - TT3K UNRAVELLING THE ROLE OF PROTEIN KINASES IN LEISHMANIA DIFFERENTIATION
Baker, Nicola Catta-Preta, Carolina; Neish, Rachel; Powell, Ben; Ferreira, Eliza; Geoghegan, Vincent; Newling, Katherine; Pitchford, John; Mottram, Jeremy (University of York)

10 A 'HUB AND SPOKE' model for the nuclear lamina in trypanosomes
Padilla-Mejia, Norma E; Koreny, Ludek; Zoltner, Martin; Field, Mark (University of Dundee)

11 A fluorescent reporter strain designed to screen for factors that initiate surface remodeling in the African trypanosome
Schulz, Danae; Walsh, Matty; Naudzius, Ellie (Harvey Mudd College)

12 A heterodimer of a kinetoplastid-specific kinesin and its catalytically inactive partner associates with the microtubule quartet of Trypanosoma brucei
Hashimi, Hassan; Müller, Nora; Kaltenbrunner, Sabine (Faculty of Science, University of South Bohemia, Ceské Budejovice, Czechia); Varga, Vladimír (Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czechia); Lukeš, Julius (Institute of Parasitology, Biology Center, Czech Academy of Sciences, Ceské Budejovice, Czechia)

13 A new type of extended-synaptotagmin (E-Syt) in Trypanosomes
Stepinac, Emma; Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna); He, Cynthia (Department of Biological Science, National University of Singapore); Bonhivers, Melanie; Robinson, Derrick R. (CNRS, Microbiologie Fondamentale et Pathogénicité); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna)

14 A single dose of antibody-drug conjugate cures a stage 1 model of African trypanosomiasis.
MacGregor, Paula; Gonzalez-Munoz, Andrea; Jobe, Fatoumatta (MedImmune); Taylor, Martin (London School of Hygiene and Tropical Medicine); Rust, Steven; Sandercock, Alan (MedImmune); Macleod, Olivia (University of Cambridge); Van Bocxlaer, Katrien; Francisco, Amanda (London School of Hygiene and Tropical Medicine); D'Hooge, Francois; Tiberghien, Arnaud; Barry, Conor; Howard, Philip (Spirogen); Higgins, Matthew (University of Oxford); Vaughan, Tristan; Minter, Ralph (MedImmune); Carrington, Mark (University of Cambridge)

15 Absence of hydrodynamic flow mediated sorting of surface bound complexes in non-African trypanosomes
Hambleton, Isobel Kelly, Steven (University of Oxford); Carrington, Mark (University of Cambridge)

16 Assessing the structure and function of trypanosome lytic factors in human breast milk
Ebenezer, Karen; Raper, Jayne (CUNY Hunter College, Department of Biological Sciences); Thomson, Russel; Verdi, Joseph (CUNY Hunter College)

17 Blastocrithidia, a trypanosomatid with all three stop codons reassigned
Lukes, Julius); Nenarokova, Anna; Zahonova, Kristina; Horakova, Eva; Kachale, Ambar; Nenarokov, Serafim (Biology Centre); Yurchenko, Vyacheslav (University of Ostrava); Paris, Zdenek (Biology Centre)

18 Cas9 editing for precision dissection of high priority anti-trypanosomal drug targets

Rico, Eva; Wall, Richard; Roberts, Adam; Lukac, Iva; Zuccotto, Fabio; Gilbert, Ian; Fairlamb, Alan; Wyllie, Susan; Horn, David (Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK)

19 Characterization of proline transporter encoded by the AAT7 gene family in Trypanosoma brucei

Haindrich, Alexander C.; Wirdnam, Corina; Niederhauser, Isabel; Rentsch, Doris (IPS, Univeristy of Bern, Bern, CHE)

20 Characterizing the role of TbAMPK and TbTOR in nutrient signaling in the African trypanosome

Jones, Jessica A; Morris, James (Clemson University)

21 Conditional gene ablation with DiCre and CRISPR/Cas9 reveals the involvement of homologous recombination factors in DNA replication and genome variability in Leishmania

Damasceno, Jeziel; Reis-Cunha, Joao (Universidade Federal de Minas Gerais); Crouch, Kathryn (University of Glasgow); Bartholomeu, Danielle (Universidade Federal de Minas Gerais); McCulloch, Richard (University of Glasgow, Glasgow, United Kingdom)

22 Control of RNA editing fidelity in trypanosomes

Meehan, Joshua; Kumar, Vikas (Texas A&M University, Biochemistry); Gulati, Shelly (University of Oklahoma Health Sciences Center); Doharey, Pawan (Texas A&M University, Biochemistry); Ivens, Al (University of Edinburgh, Institute of Immunology); Mcdermott, Suzanne (Center for Infectious Disease Research); Schnaufer, Achim (University of Edinburgh, Institute of Immunology); Stuart, Ken (Center for Infectious Disease Research); Mooers, Blaine (University of Oklahoma Health Sciences Center); Cruz-Reyes, Jorge (Texas A&M University, Biochemistry)

23 Controlling the surface proteome: A novel nndosomal tetratricopeptide-repeat protein interacts with deubiquitylase TbUsp7 and the TbSkp1-like protein in Trypanosoma brucei.

Yamada, Kayo Canavante, Ricardo; Zoltner, Martin; Field, Mark (University of Dundee)

24 Correlating Trypanosoma brucei localisation, tryptophan metabolism and behavioural changes in the rodent model of HAT

Fathallah N.A.1, Dawson N.1, Urbaniak M.D.1
1Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, United Kingdom;

25 Cyclophilin 19 a new player in oxidative stress during Trypanosoma cruzi infection.

Pedroso dos Santos, Gregory Midori Abukawa, Fernanda (Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil); Maria Alcântara, Laura (Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil); Silvio Moretti, Nilmar (Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil); S. Mcgwire, Bradford (Division of Infectious Diseases/Center for Microbial Interface Biology, The Ohio State University); Schenkman, Sergio (Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil)

26 Deciphering the molecular machinery of the antigenic variation regulator DOT1B

Eisenhuth, Nicole; Vellmer, Tim (Department of Cell and Developmental Biology, Würzburg, Germany); Dejung, Mario; Butter, Falk (Institute of Molecular Biology, Mainz, Germany); Janzen, Christian J (Department of Cell and Developmental Biology, Würzburg, Germany)

27 Deep mutational scanning reveals developmentally distinct functions for KREPB4 in RNA editing

McDermott, Suzanne M; Stuart, Kenneth (Seattle Children's Research Institute)

28 Different extracellular vesicles are secreted from Trypanosoma brucei under starvation, heat shock and drugs-biochemical and functional analyses

Michaeli, Shulamit; Kennan, Sriram; Eliaz, Dror (Bar-Ilan University)

29 Discovery of a kinetochore-based ATM/ATR-independent DNA damage checkpoint in Trypanosoma brucei

Zhou, Qing Pham, Kieu T. M.; Hu, Huiqing; Kurasawa, Yasuhiro; Li, Ziyin (University of Texas Health Science Center)

30 Divergent RNase III domains in KREPB6, KREPB7, and KREPB8 are essential for editing in both procyclic and bloodstream form Trypanosoma brucei

Carnes, Jason; McDermott, Suzanne; Stuart, Kenneth (Seattle Children's Research Institute)

31 Editing of Minimally Edited RNAs in Trypanosoma brucei

Tylec, Brianna L.; Simpson, Rachel; Chen, Runpu; Sun, Yijun; Read, Laurie (University at Buffalo)

32 Effects of small molecule inhibitors of DNA break repair on Trypanosoma brucei
Hovel-Miner, Galadriel; Wallin, Stephen; Howard, J. Nataile (George Washington University)

33 END-seq: a new whole-genome approach for the analysis of DNA double-stranded breaks in the African Trypanosome
Sciascia, Nicholas Quinn, McKenzie (George Washington University); Wu, Wei (National Cancer Institute, NIH); Hovel-Miner, Galadriel (George Washington University)

34 Epigenetic regulation at replicative and non-replicative forms of Trypanosoma cruzi
P.C. da Cunha, Julia Poubel, Saloe Bispo (Butantan Institute); Lima, Alex Ranieri Jerônimo (UFPA); Roson, Juliana Nunes; Araujo, Christiane (Butantan Institute); Gonçalves, Evonildo (UFPA); Masotti, Cibele; A.F. Galante, Pedro (Sirio Libanes Hospital); Elias, Maria Carolina (Butantan Institute)

35 Evolution of metabolic and molecular features in Kinetoplastea revealed by comparative genomics
Butenko, Anzhelika Flegontova, Olga; Horák, Aleš (Institute of Parasitology, Biology Centre, Ceské Budejovice, Czech Republic); Hampl, Vladimír (Faculty of Science, Charles University, Prague, Czech Republic,); Keeling, Patrick (Department of Botany, University of British Columbia, Vancouver, Canada,); Gawryluk, Ryan (Department of Biology, University of Victoria, Victoria, Canada,); Tikhonenkov, Denis (Department of Botany, University of British Columbia, Vancouver, Canada; Institute for Biology of Inland Waters, Russian Academy of Sciences,); Flegontov, Pavel (Institute of Parasitology, Biology Centre, Ceské Budejovice, Czech Republic; Faculty of Science, University of Ostrava, Ostrava, Czech Republic,); Lukeš, Julius (Institute of Parasitology, Biology Centre, Ceské Budejovice; Faculty of Science, University of South Bohemia, Ceské Budejovice, Czech Republic,)

36 Flow Cytometry-Assisted Fluorescent Barcoding for Simultaneous Interrogation of Multiple Metabolites in Live Kinetoplastid Parasites
Vance, Jacob Voyton, Charles (Clemson University); Christensen, Kenneth A. (Brigham Young University, Provo, UT)

37 Functional interplays between Polo-like kinase and cytokinesis regulatory proteins in Trypanosoma brucei
Lee, Kyu j. Kurasawa, Yasuhiro; An, Tai; Li, Ziyin (University of Texas)

38 Functionally mapping the evolutionary diversification of Trypanosoma congolense and Trypanosoma brucei using spatial proteomics
Moloney, Nicola Barylyuk, Konstantin; Lilley, Kathryn; Waller, Ross; MacGregor, Paula (University of Cambridge)

39 Glycosome heterogeneity in Trypanosoma brucei is likely a function of organelle maturation and functional specialization
Crowe, Logan Wilkinson, Christina; Knight, Emily; Morris, Meredith (Clemson University)

40 Identification and characterization of the early acting glycosome biogenic protein PEX3 of Trypanosoma brucei-a target for drug development against the trypanosomatid diseases

Banerjee, Hiren Knoblach, Barbara; Rachubinski, Richard A. (University of Alberta)

41 IDENTIFYING CRITICAL INTERACTIONS IN THE UNIQUE TRYPANOSOMA BRUCEI 5S RIBONUCLEOPROTEIN COMPLEX AND THEIR ROLE IN RIBOSOME BIOGENESIS

Jaremko, Daniel Ciganda, Martin; Williams, Noreen (University at Buffalo, Jacobs School of Medicine and Biomedical Sciences)

42 Insight into the active purinome of Leishmania donovani: Identification of potential novel purine-binding proteins

Leclercq, Olivier Dingli, Florent (Institut Curie, Laboratoire de spectrométrie de masse protéomique, Paris); Criscuolo, Alexis (Institut Pasteur, Centre de bioinformatique, bio-statistique et biologie intégrative, Paris); Guglielmini, Julien (Institut Pasteur, Centre de bioinformatique, bio-statistique et biologie intégrative, Par); Arras, Guillaume; Loew, Damarys (Institut Curie, Laboratoire de spectrométrie de masse protéomique, Paris); Rachidi, Najma; Späth, Gerald (Institut Pasteur and INSERM U1201, unité de parasitologie moléculaire et signalisation, Paris)

43 Interferon-gamma mediates inhibition of Plasmodium hepatic infection by Trypanosoma brucei

Sanches-Vaz, Margarida Luis, Rafael; Temporão, Adriana; Mendes, António M.; Goellner, Sarah; Carvalho, Tânia; Prudêncio, Miguel; Figueiredo, Luisa M. (Instituto de Medicina Molecular)

44 Loss of the haptoglobin-haemoglobin receptor in Trypanosoma brucei blocks life-cycle differentiation

Horakova, Eva Lacordier, Laurence; Cahuna, Paula (Laboratory of Molecular Parasitology, Institut de Biologie et de Medecince Moleculaires, Universite Libre de Bruxelles, B6041 Gosselies, Belgium); Changmai, Piya (Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceské Budejovice, Czech Republic); Sobotka, Roman (Institute of Microbiology, Czech Academy of Sciences, Trebon, Czech Republic); Lukeš, Julius (Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceské Budejovice, Czech Republic); Vanhollebeke, Benoit (Laboratory of Molecular Parasitology, Institut de Biologie et de Medecince Moleculaires, Universite Libre de Bruxelles, B6041 Gosselies, Belgium)

45 Mitochondrial Intermembrane Space Protein Tim54: A crucial component required for the import of Metabolite Carrier Proteins in Trypanosoma brucei.

Ujjal K. Singha, Anuj Tripathi, Aparajita Saha, Joseph T. Smith, Tanusree Singha, and Minu Chaudhuri. Meharry Medical College, Nashville TN 37208. [*Corresponding author]*

46 Modeling of Trypanosoma brucei population dynamics in mice reveals slow-growing parasites in adipose tissue

Figueiredo, Luisa M Trindade, Sandra; Sequeira, Mariana (Instituto de Medicina Molecular); Dejung, Mario; Bento, Fabio (Institute of Molecular Biology); Pinto-Neves, Daniel (Instituto Gulbenkian de Ciencia); Butter, Falk (Institute of Molecular Biology); Bringaud, Frederic (Universite de Bordeaux); Gjini, Erida (Instituto Gulbenkian de Ciencia)

47 Molecular and functional characterisation of Leishmania casein kinase 1 provides evidences for a role in endocytosis.

Martel, Daniel Ndiaye, Paya; Pine, Stewart; Späth, Gerald; Rachidi, Najma (Institut Pasteur and INSERM U1201, Unité de Parasitologie moléculaire et Signalisation, Paris)

48 New approaches to studying the GPI biosynthesis pathway in T. brucei: uncovering the missing links.

Ji, Zhe Tinti, Michele; Duncan, Samuel; Guther, Lucia; Ferguson, Micheal (University of Dundee)

49 Obtaining a high-quality, phased genome of Trypanosoma brucei and what to do with it

Brink, Benedikt Cosentino, Raul; Müller, Laura; Siegel, Nicolai (LMU Munich)

50 Non-invasive monitoring of drug action: Exploring a new assay design for Chagas' disease in vitro drug discovery

Fesser, Anna Braissant, Olivier (Department of Biomedical Engineering, University Basel, Switzerland); Rocchetti, Romina (Swiss Tropical & Public Health Institute, University of Basel, Switzerland); Olmo, Francisco; Kelly, John (London School of Hygiene and Tropical Medicine, London, UK); Mäser, Pascal; Kaiser, Marcel (Swiss Tropical & Public Health Institute, University Basel, Switzerland)

51 Orally delivered gold(I) complexe combined with miltefosine reduces treatment scheme by half on experimental leishmaniasis

Monte-Neto, Rubens Tunes, Luiza (Instituto René Rachou - Fiocruz Minas, Belo Horizonte, Brazil); Garcia, Adriana (Universidade Federal de Juiz de Fora, Juiz de Fora, Brasil); Schmidtz, Vinicius; dos Santos, Helio (Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil); Silva, Heveline; Frézard, Frédéric; Barros, André Luis (Universidade Federal de Minas Gerais, Belo Horizonte, Brazil)

52 Population genomic analysis of Leishmania infantum in Piauí, Brazil

Forrester, Sarah Carnielli, Juliana (York Biomedical Research institute, Department of Biology, University of York, United Kingdom); Costa Silva, Vladimir (Laboratório de Pesquisas em Leishmanioses, Instituto de Doenças Tropicais Natan Portella, Universidade Federal do Piauí, Teresina-PI, Brazil); James, Sally (1. Technology facility, Genomics facility, University of York, United Kingdom); Costa, Dorcas; Costa, Carlos (Laboratório de Pesquisas em Leishmanioses, Instituto de Doenças Tropicais Natan Portella, Universidade Federal do Piauí, Teresina-PI, Brazil); Mottram, Jeremy; Jeffares, Daniel (1. York Biomedical Research institute, Department of Biology, University of York, United Kingdom)

POSTERS: Session B
Monday 7:00 pm
04/29/19

53 Proteomics in kinetoplast species
Butter, Falk (Institute of Molecular Biology, Mainz, Germany)

54 RNA quality control in mitochondria of Trypanosoma brucei
Afasizheva, Inna (Boston University)

55 RNA viruses in trypanosomatids
Yurchenko, Vyacheslav Grybchuk, Danyil; Kostygov, Alexei; Macedo, Diego (Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic); Lukeš, Julius (Institute of Parasitology, Biology Centre, and Faculty of Science, University of South Bohemia, C?eské Bude?jovice (Budweis), Czech Republic)

56 Genome-wide screening for modifiers of an anaphase entry mutant provides insights into the trypanosome kinetochore regulatory network
Brusini, Lorenzo Wickstead, Bill (University of Nottingham)

57 Role of the telomere binding protein TbUMSBP2 in chromatin remodeling in trypanosomes
Soni, Awakash Klebanov-Akopyan, Olga; Shlomai, Joseph (Hebrew University, Jerusalem, Israel)

58 Stage-specific function of Alba3 in Trypanosoma brucei
Bevkal Subramanyaswamy, Shubha Heller, Manfred (Head of Proteomics & Mass Spectrometry Core Facility, University of Bern); Naguleswaran, Arunasalam; Roditi, Isabel (Institute of Cell Biology, University of Bern)

59 Structural and Mechanistic insight into Trypanosoma brucei Telomerase RNA
Dey, Abhishek Saha, Arpita; Li, Bibo (Cleveland State University); Chakrabarti, Kausik (University of North Carolina at Charlotte)

60 Structure of T. brucei CC2D suggests an auto-regulatory membrane binding of its C2 domain
Dong, Gang Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)

61 Systematic Analysis of potential histone mark writers, readers and erasers in Trypanosoma brucei
Carloni, Roberta Staneva, Desislava; Tong, Pin; Auchynnikava, Tatsiana; Matthews, Keith; Allshire, Robin (University of Edinburgh)

62 T. brucei RAP1 has a DNA binding activity that is important for VSG monoallelic expression.
Afrin, Marjia Gaurav, Amit (Cleveland State University); Yang, Xian (The Hong Kong Polytechnic University); Sandhu, Ranjodh (Cleveland State University); Zhao, Yanxiang (The Hong Kong Polytechnic University); Li, Bibo (Cleveland State University)

63 The 'essential genome' differs substantially between monomorphic and pleomorphic trypanosomes

D'Archivio, Simon Whipple, Sarah (University of Nottingham); Trindade, Sandra; Figueiredo, Luisa (Instituto de Medicina Molecular); Wickstead, Bill; Gadelha, Catarina (University of Nottingham)

64 The hook complex-associated protein BOH1 in Trypanosoma brucei cooperates with Polo-like kinase to regulate flagellum inheritance and cytokinesis initiation

Li, Ziyin Pham, Kieu; Zhou, Qing; Kurasawa, Yasuhiro (University of Texas Medical School at Houston)

65 The impact of novel telomere-associated protein complexes on VSG expression site regulation in Trypanosoma brucei

Weisert, Nadine Luko, Katarina; Dejung, Mario; Butter, Falk (Institute of Molecular Biology (IMB), Mainz, Germany); Janzen, Christian J. (Department of Cell and Developmental Biology, Würzburg, Germany)

66 The kDNA replication apparatus: novel components and ancestry from mobile elements

KRISHNAN, ARUNKUMAR Burroughs,, A. Maxwell; M. Iyer, Lakshminarayan; L., Aravind (NIH/NLM/NCBI)

67 The Leishmania infantum Miltefosine Sensitivity Locus

Brambilla Trindade Carnielli, Juliana Forrester, Sarah; Jeffares, Daniel C.; Davey, John (York Biomedical Research Institute, Department of Biology, University of York, UK); Costa, Carlos Henrique Neri (Laboratório de Pesquisas em Leishmanioses, Instituto de Doenças Tropicais Natan Portella, Universidade Federal do Piauí, Brazil); Mottram, Jeremy C. (York Biomedical Research Institute, Department of Biology, University of York, UK)

68 The microtubule-associated protein PAVE1 regulates the dynamics of the subpellicular microtubule array in Trypanosoma brucei

Sinclair-Davis, Amy Sladewski, Thomas; de Graffenried, Christopher (Brown University)

69 The Trypanosoma brucei infectious form mitochondrion is capable of ATP generation

Dolezelova, Eva Taleva, Gergana (1 Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic 2 Faculty of Science, University of South Bohemia, Ceske Budejovice,); Panicucci, Brian (Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic); Hierro-Yap, Carolina (1 Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic 2 Faculty of Science, University of South Bohemia, Ceske Budejovice,); Husova, Michaela (Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic); Pineda, Erika; Bringaud, Frederic (University of Bordeaux, Bordeaux, France); Zíková, Alena (1 Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic 2 Faculty of Science, University of South Bohemia, Ceske Budejovice,)

70 Trafficking itinerary of aquaglyceroporin 2 in T. brucei: new insights into the mode of action of pentamidine
Quintana, Juan Field, Mark (University of Dundee)

71 Transcription activity contributes to the firing of non-constitutive origins in Trypanosoma brucei maintaining the robustness of the S phase duration
da Silva, Marcelo Santos Cayres-Silva, Gustavo; Vitarelli, Marcela; Marin, Paula (Laboratório Especial de Ciclo Celular, Center of Toxins, Immune Response and Cell Signaling (CeTICS), Instituto Butantan, São Paulo, Brazil); Hiraiwa, Priscila (Plataforma de citometria de fluxo, Instituto Carlos Chagas, FIOCRUZ, Paraná, Brazil); Araújo, Christiane (Laboratório Especial de Ciclo Celular, Center of Toxins, Immune Response and Cell Signaling (CeTICS), Instituto Butantan, São Paulo, Brazil); Ávila, Andrea (Laboratório de Regulação da Expressão Gênica, Instituto Carlos Chagas, FIOCRUZ, Paraná, Brazil); McCulloch, Richard (The Wellcome Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom); Reis, Marcelo; Elias, Maria Carolina (Laboratório Especial de Ciclo Celular, Center of Toxins, Immune Response and Cell Signaling (CeTICS), Instituto Butantan, São Paulo, Brazil)

72 TRANSCRIPTIONAL CONTROL OF MITOCHONDRIAL GENE EXPRESSION IN TRYPANOSOMA BRUCEI
Afasizhev, Ruslan (Boston University)

73 Transfer of surface functionality between trypanosome species
Aroko, Erick Jones, Nicola; Engstler, Markus (Würzburg University)

74 TriTrypDB: the functional genomics resource for kinetoplastida
Harb, Omar S. Crouch, Kathryn (University of Glasgow); Roos, David (University of Pennsylvania); Hertz-Fowler, Christiane (University of Liverpool)

75 Trypanosome Lytic Factor mediated Protection against Leishmania sp.
Pant, Jyoti Keceli, Mert (Hunter college); Verdi, Joseph (The Graduate Center); Raper, Jayne (Hunter College)

76 Basalin, a conserved yet evolutionarily unconstrained flagellum protein. Implications for analysis of the Kinetoplastid genomes
Dean, Samuel Moreira-Leite, Flavia; Gull, Keith (University of Oxford)

77 Ubiquitin in the trypanosomes: the Cullin-RING complexes
Canavate del Pino, Ricardo Ono, Kayo (University of Dundee); Zoltner, Martin (Charles University of Prague); Field, Mark C. (University of Dundee)

78 Unravelling the mode of action of two organometallic anti-T. cruzi compounds
Mosquillo, Florencia Smircich, Pablo (Facultad de Ciencias, Universidad de la República); Gambino, Dinorah (Facultad de Química, Universidad de la República); Garat, Beatriz; Pérez-Díaz, Leticia (Facultad de Ciencias, Universidad de la República)

79 Widespread roles for the ATR kinase in maintenance of the nucleus and genome of Trypanosoma brucei
Black, Jennifer Ann Crouch, Kathryn; Lemgruber, Leandro; Briggs, Emma; Lapsley, Craig (University of Glasgow); Mottram, Jeremy C (University of York); Tosi, Luiz R O (Universidade de São Paulo); McCulloch, Richard (University of Glasgow)

80 A CRISPR/Cas9/riboswitch-based method for downregulation of gene expression in Trypanosoma cruzi
Lander, Noelia M. Cruz-Bustos, Teresa; Docampo, Roberto (University of Georgia)

81 A targeted RNAi screen identifies genes that play a role at different stages of Trypanosoma brucei metacyclogenesis.
Nkouawa, Agathe Y. Toh, Justin; Rojas-Sánchez, Saúl; Shi, Huafang; Lei, Yuling; G. Kolev, Nikolay; Tschudi, Christian (Yale School of Public Health)

82 Activity of fire ant venom alkaloids against Trypanosoma cruzi: quest for an effective alternative therapy
Heise, Norton Costa Silva, Rafael; Fox, Eduardo (Universidade Federal do Rio de Janeiro); Gomes, Fabio (NIAID-NIH); Feijó, Daniel; Ramos, Isabela (Universidade Federal do Rio de Janeiro); Koeller, Carolina (SUNY - University at Buffalo); Costa, Tatiana; Rodrigues, Nathalia; Lima, Ana Paula; Atella, Georgia; Miranda, Kildare (Universidade Federal do Rio de Janeiro); Schoijet, Alejandra; Alonso, Guillermo (INGEBI-CONICET); Machado, Ednildo (Universidade Federal do Rio de Janeiro)

83 Addressing the choices made in VSG switching by single cell RNA sequencing
Subota, Ines Wange, Lucas; Brink, Benedikt; Luzak, Vanessa; Enard, Wolfgang; Siegel, Nicolai (LMU Munich)

84 Analysis of molecules released by African trypanosomes and their role in parasite transmission
Tettey, Mabel Deladem Rojas, Federico; Matthews, Keith R. (School of Biological Sciences, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland)

85 Analyzing the basic function of Trypanosoma brucei's two introns
Srivastava, Ankita O'Connor, Zachary; Günzl, Arthur (UConn Health)

86 Building a Genetic Toolkit for Crithidia fasciculata
Malfara, Madeline DiMaio, John; Povelones, Megan (Penn State Brandywine)

87 Characterization of Deubiquitinases involved in endocytic pathway in T. cruzi
SOUZA-MELO, NORMANDA BRITO SILVA, NATHALIA; PEDROSO SANTOS, GREGORY (FEDERAL UNIVERSITY OF SAO PAULO); MARIA ALCANTARA, LAURA (UNIVERSITY OF SAO PAULO); MUNIZ MALVEZZI, AMARANTA; SCHENKMAN, SERGIO (FEDERAL UNIVERSITY OF SAO PAULO)

88 CHARACTERIZATION OF THE DNA DAMAGE RESPONSE KINASES ATR AND ATM IN LEISHMANIA MAJOR
Almeida da Silva, Gabriel Lamak Bastos, Matheus S; Virgilio, Stela; Black, Jennifer Ann; McCulloch, Richard; Orsini Tosi, Luiz Ricardo

89 Characterization of wild trypanosome coats using spliced-leader sequence enrichment and RNA-Seq
So, Jaime Otieku Oworae, Kwadwo; Manful Gwira, Theresa (University of Ghana); Mugnier, Monica (Johns Hopkins Bloomberg School of Public Health)

90 Controlling the surface proteome: A novel nndosomal tetratricopeptide-repeat protein interacts with deubiquitylase TbUsp7 and the TbSkp1-like protein in Trypanosoma brucei.
Yamada, Kayo Canavante, Ricardo; Zoltner, Martin; Field, Mark (University of Dundee)

91 Damage and Antigenic Variation in African trypanosomes.
McLaughlin, Emilia Chaze, Thibault; Matondo, Mariette (Institut Pasteur Paris); Urbaniak, Michael (Lancaster University, UK); Glover, Lucy (Institut Pasteur Paris)

92 Depleting extracellular Ca2+ leads to a decrease in cytotoxicity due to enhanced Trypanosome-protective variants of APOL1
Khalizova, Nailya Shirinyan-Tuka, Lilit; Giovinazzo, Joe; Thomson, Russell; Raper, Jayne (Hunter College CUNY)

93 Detection of Trypanosoma cruzi Secreted Antigens in Blood of Infected Hosts
Acosta, David Nagarkatti, Rana (CBER, Food and Drug Administration); Fortes de Araujo, Fernanda; Teixeira-Carvalho, Andréa (Instituto René Rachou, Fiocruz); Debrabant, Alain (CBER, Food and Drug Administration))

94 Development of the protein kinase, AEK1, in Trypanosoma brucei as a promising drug target
Jensen, Bryan Parsons, Ben; Booster, Nicholas; Dean, Marissa (Seattle Children's Research Institute); Vidadala, Rao; Maly, Dustin (University of Washington); Parsons, Marilyn (Seattle Childrens Research Institute)

95 Developmental Regulation of CYb and COIII mRNA Editing Occurs by Distinct Mechanisms in Trypanosoma brucei
Smith Jr., Joseph Tylec, Brianna; Read, Laurie (University of Buffalo)

96 Dissecting the Functions of a Divergent Mitochondrial DNA Polymerase in Trypanosoma brucei
Delzell, Stephanie Nelson, Scott (Iowa State University); Klingbeil, Michele (University of Massachusetts, Amherst)

97 DNA Damage Response in African Trypanosomes
Sima, Nuria Coron, Ross; Glover, Lucy (Institut Pasteur)

98 DNA Replication in Leishmania – an odd start?
Marques, Catarina Damasceno, Jeziel; McCulloch, Richard (Wellcome Centre for Integrative Parasitology, University of Glasgow)

99 Expression of mutant variant surface glycoprotein in bloodstream form Trypanosoma brucei triggers a severe growth defect
Ooi, Cher-Pheng Schwede, Angela; Carrington, Mark (Cambridge University); Rudenko, Gloria (Imperial College)

100 Fatty acid synthesis and lipid homeostasis in T. brucei: a response to ACC depletion
Saliutama, Joshua Vigueira, Patrick; Paul, Kimberly (Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University)

102 Genetic tools for efficient inducible expression and high-complexity library production in Trypanosoma congolense
Awuah-Mensah, Georgina Steketee, Pieter; Morrison, Liam (University of Edinburgh); Gadelha, Catarina; Wickstead, Bill (University of Nottingham)

103 Glycosylation of transferrin receptor (TfR) in bloodstream form Trypanosoma brucei: steric constraints control GPI-glycan modification
Koeller, Carolina M. Tiengwe, Calvin (Imperial College); Schwartz, Kevin J. (University of Wisconsin-Madison); Bangs, James D. (University at Buffalo)

104 How does a trypanosome change its spots? Decrypting immune avoidance in human kinetoplastids
Warren, Felix Llwellyn, Martin; McCulloch, Richard; Burchmore, Richard (The University of Glasgow); Gadelha, Catarina (The University of Nottingham)

POSTERS: Session C
Tuesday 7:00 pm

04/30/19

105 – TT5H Characterising Leishmania kinetochores with XL-BioID
Geoghegan, Vincent Jones, Nathaniel; Dowle, Adam; Larson, Tony; Mottram, Jeremy (University of York)

106 – TT5I Identifying novel factors associated with trypanosome DNA replication forks using nascent DNA proteomics
Rocha-Granados, Maria Bermudez, Yahaira; Dodard, Garvin (University of Massachusetts); Gunzl, Arthur (UConn Health); Klingbeil, Michele M. (University of Massachusetts, Amherst, Amherst, MA)

107 – TT6H An ORF-based whole-genome gain-of-function library for Trypanosoma brucei
Quinn, McKenzie Gomez, Stephanie (The George Washington University); Schultz, Danae (Harvey Mudd College); Kim, Hee-Sook (Cleveland State University); Hovel-Miner, Galdriel (The George Washington University)

108 – TT6I UTR-seq: hundreds of new regulatory 3'-untranslated regions in African trypanosomes
Trenaman, Anna Wall, Richard; Horn, David (University of Dundee)

109 – TT6K Characterization of putative chromo- and SET-domain transcription regulators in Trypanosoma brucei
Staneva, Desislava Carloni, Roberta; Auchynnikava, Tatsiana; Tong, Pin; Matthews, Keith; Allshire, Robin (The University of Edinburgh)

110 – TT7L Intriguing roles assigned to Leishmania eIF4E paralogs by structure-function studies and CRISPR-Cas 9-mediated knockout approach
Shapira, Michal Tupperwar, Nitin; Shrivastava, Rohit; Kamus-Elimeleh, Dikla; Orr, Irit (Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel); Meleppattu, Shimi; Leger-Abraham, Melissa (Microbiology Department, Blavatnik Institute, Harvard Medical School, Boston, MA); Wagner, Gerhard (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA)

111 Overexpression of a Trypanosoma cruzi mitochondrial ribonuclease affects cell morphology and differentiation
Zimmer, Sara Ramirez-Barrios, Roger (Department of Biomedical Sciences, University of Minnesota Medical School, Duluth campus, Duluth, Minnesota, USA.)

112 Potential usage of extracellular amastigotes of Trypanosoma cruzi as a temporal axenic culture for transient gene expression, drug sensitivity assay, and CRISPR/Cas9-mediated gene knockout.
Takagi, Yuko Akutsu, Yukie; Doi, Motomichi; Furukawa, Koji

113 Rapid block of RNA splicing by chemical inhibition of analog-sensitive CRK9 in Trypanosoma brucei
Gosavi, Ujwala A.

114 Regulation of expression of the RNA-binding protein RBP10 in Trypanosoma brucei
Bishola, Tania LIU, BIN (ZMBH); TERRAO, MONICA (UNIKLINIK KOLN); CLAYTON, CHRISTINE (ZMBH, UNIVERSITY OF HEIDELBERG)

115 RNA-binding protein 5 (RBP5) plays a role in cell cycle progression in Trypanosoma brucei
Anaguano, David Klingbeil, Michele M. (University of Massachusetts, Amherst, Amherst, MA)

116 Specific roles for homologous recombination in maintenance of the Trypanosoma brucei subtelomeric VSG (pseudo)gene archive
Krasilnikova, Marijab Crouch, Kathryn; McCulloch, Richard (Wellcome Centre for Integrative Parasitology / University of Glasgow, UK)

117 SUMO chain mutants promote oscillating parasitemia and extended host survival during T. brucei infection in mice
Iribarren, Paula Ana Coria, Lorena; Di Marzio, Lucía; Berazategui, María Agustina (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina); Saura, Andreu (Instituto de Parasitología y Biomedicina "López-Neyra", CSIC (IPBLN-CSIC), 18016 Granada, Spain); Cassataro, Juliana (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina); Navarro, Miguel (Instituto de Parasitología y Biomedicina "López-Neyra", CSIC (IPBLN-CSIC), 18016 Granada, Spain); Alvarez, Vanina Eder E. (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina, San Martin, Argentina)

118 Systems-level Analyses to Elucidate Key Purine Stress Response Candidates in Leishmania
Carter, Nicola Yates, Phillip (Oregon Health & Science University); Shepard, Emily; Suchovsky, Skyler (Pacific University School of Pharmacy); Wilmarth, Phillip; David, Larry (Oregon Health & Science University)

119 TbmtHMG44 is a novel HMG-box protein involved in kDNA maintenance in Trypanosoma brucei
Ochsenreiter, Torsten Hoffmann, Anneliese; Baudouin, Hélène; Amodeo, Simona (University of Bern); Gull, Keith (University of Oxford); Zuber, Benoît (University of Bern); Varga, Vladimir (Institute of Molecular Genetics Prague)

120 The Hunt for the Trypanosoma brucei VSG GPI-anchor sn1 Remodelase
Poudyal, Nava Paul, Kimberly (Clemson University)

121 THE IMPACT OF MIXED SPECIES INFECTIONS ON TRYPANOSOME VIRULENCE AND TRANSMISSION
Venter, Frank Ivens, Alasdair (Centre for Immunity, Infection and Evolution, Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh,); Matthews, Keith R. (Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh E)

122 The nuclear export receptors TbMex67-TbMtr2 are required for ribosome biogenesis in Trypanosoma brucei.
Ciganda, Martin Rink, Constance; Williams, Noreen (University at Buffalo)

123 The RNA-binding proteins RBP7-A and B play distinct roles during the acquisition of infectivity in Trypanosoma brucei
Rojas Sanchez, Saul Tschudi, Christian (Yale School of Publiic Health)

124 Tidying up the nucleus – which molecular factors build the compartments of VSG gene regulation?
Luzak, Vanessa, Brink, Benedikt; Siegel, Nicolai (LMU Munich)

125 Transcriptomic profiling of cellular and axenic amastigotes of T. cruzi
Smircich, Pablo Bilbao, Lucia (Instituto de Investigaciones Biológicas Clemente Estable / Facultad de Ciencias); Hernández, Fabricio (Facultad de Ciencias); Sotelo-Silveira, José (Instituto de Investigaciones Biológicas Clemente Estable / Facultad de Ciencias); Garat, Beatriz; Pérez-Díaz, Leticia (Facultad de Ciencias)

126 Trypanosoma brucei TIF3 is a novel telomere protein that is essential for cell viability and affects VSG switching
Schnur, Brittny, Li, Bibo (Cleveland State University, Cleveland, OH)

127 Trypanosome differentiation
Matthews, Keith R.(University of Edinburgh)

128 TrypSpotting: Identifying Lipid Droplet Proteins in Trypanosoma brucei
Raja, Sripriya

129 Ubiquitin Conjugating Enzymes in Leishmania: When do they work, with whom, and can they be targeted?
Harris, Daniel

130 Variant Surface Glycoprotein Expression in Tissue-resident Trypanosoma brucei
Beaver, Alexander, Zhang, Lucy (Johns Hopkins School of Public Health); Bobb, Bryce; Rijo-Ferreira, Filipa; Figueiredo, Luisa ; Mugnier, Monica (Johns Hopkins School of Public Health)

131 VEX1 regulates metacyclic VSG expression in insect stage trypanosomes
Tihon, Eliane Dujeancourt- Henry, Annick; Glover, Lucy (Institut Pasteur)

132 VSG length dependent growth rate in Trypanosoma brucei
Scheidt, Viktor Horn, David (Dundee University)

133 What happens inside the nucleus when trypanosomes switch expression of VSGs?
Rabuffo, ClaudiaU (LMU Munich)

134 Whole genome RNAi library screens identify repressors of metacyclic VSG expression site transcription in bloodstream form T. brucei
Davies, Carys Sioutas, George; Sidhu, Haneesh; Hall, Belinda (Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, London); Wickstead, Bill (School of Life Sciences, University of Nottingham, UK); Alsford, Sam (London School of Hygiene and Tropical Medicine, London); Rudenko, Gloria (Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, London)

135 ZC3H20 and ZC3H21 in Trypanosoma brucei: the targets and functions.
Liu, Bin Marucha, Kevin; Bajak, Kathrin; Nascimento, Larissa; Clayton, Christine (Zentrum für Molekulare Biologie der Universität Heidelberg)

136 Identification and function of the VSG transcript-bound proteome: methodological development and preliminary findings
ERBEN, Esteban D. STROHMENGER, Soren (DKFZ); CLAYTON, Christine (ZMBH); PAPAVASILIOU, Nina (DKFZ)

137 Identification of Biomarkers of Trypanosoma cruzi Infected Cells
Acharyya, Nirmallya Serna, Carylinda (National Cancer Institute, NIH); Silberstein, Erica; Acosta, David; Nagarkatti, Rana; Debrabant, Alain (Food and Drug Administration)

138 Identification of kinetoplastid parasite glucose uptake inhibitors
Call, Daniel Vance, Jacob (Brigham Young University); Voyton, Charles (Clemson University); Choi, Jongsu (Brigham Young University); Werbovetz, Karl (The Ohio State University); Golden, Jennifer (University of Wisconsin-Madison); Morris, James (Clemson University); Christensen, Kenneth (Brigham Young University)

139 Impact on virulence of flagellar pocket protein kinases in Leishmania mexicana
Neish, Rachel Mottram, Jeremy (University of York)

140 In Trypanosoma brucei, activity of fructose, 1-6, bisphosphatase, an enzyme involved in gluconeogenesis, is upregulated in high-glucose conditions
Wilkinson, Christina Morris, Meredith (Clemson University)

141 Inducible suicide system in Leishmania mexicana
Podesvova, Lucie Yurchenko, Vyacheslav (University of Ostrava, Faculty of Science, Life Science Research Centre, Czech Republic)

142 Inhibitors of CLK1 protein kinase in kinetoplastids
Bower-Lepts, Christopher Saldivia, Manuel; Mottram, Jeremy (University of York); Rao, Srinivasa (Novartis Institute of Tropical Disease)

143 Intrinsic susceptibility to Amphotericin B in clinical isolates of Leishmania subgenus Viannia.
Echeverry, Maria Franco, Carlos (Centro Dermatológico Federico Lleras Acosta); Ovalle-Bracho, Clemencia (CentroDermatológico Federico Lleras Acosta); Saavedra, Carlos (Facultad de Medicina, Universidad Nacional de Colombia)

144 Investigating ciliopathy-linked polymorphisms in IFT172 using precision Cas9 mediated genome editing in Trypanosoma brucei
Hutchinson, Sebastian Jung, Jamin; Bastin, Philippe (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, 25-28 Rue du Dr Roux, Paris, 75015 France.)

145 Investigating Dynamics in the Kinetoplastid Mitochondrial Network
DiMaio, John Malfara, Madeline; Povelones, Megan (Penn State Brandywine)

146 Investigating the Role of Potassium Ion Flow in Trypanolysis
Oprea, Yasmine Ko, Daphne (CUNY Hunter College); Verdi, Joey (CUNY Graduate Center); Raper, Jayne (CUNY Hunter College)

147 Investigation of iron superoxide dismutase role in Leishmania infantum
Santi, Ana Maria Silva, Paula; Diefenbach, Katharina (René Rachou Institute, FIOCRUZ MINAS); Beverley, Stephen (Washington University School of Medicine in St. Louis); Murta, Silvane (René Rachou Institute, FIOCRUZ MINAS)

148 KHARON interacting proteins KHAP1 and KHAP2 are essential for growth of Leishmania amastigotes
Kelly, Felice D. Hatfield, Jess (University of Notre Dame); Landfear, Scott (Oregon Health Sciences University)

149 Lysine acetylation: the rise of the rival, a perspective from Trypanosomatids
Moretti, Nilmar Barbosa Leite, Ariely; Maria dos Santos Moura, Leila; Caroline de Castro Nascimento, Ana; Maran, Suellen; Schenkman, Sergio (Federal University of Sao Paulo)

150 Natural IgM antibodies bind to Trypanosome Lytic Factor 1 to form TLF2
Savran, Michelle Verdi, Joey; Raper, Jayne (Hunter College CUNY)

151 On the way to the T. brucei Flagellar Pocket Collar

Bonhivers, Melanie Isch, Charlotte; Landrein, Nicolas (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France); Majneri, Paul; Pivovarova, Yulia; Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria); Robinson, Derrick (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)

SESSION I:
VSG
Mark Carrington, Chair.
Saturday 7:00 pm
04/27/19

1A Identification of novel ESB associated proteins in bloodstream form Trypanosoma brucei

Budzak, James Jones, Robert (Sir Alexander Fleming Building, Dept. of Life Sciences, Imperial College London, U.K.); Tschudi, Christian; Kolev, Nikolay G. (Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT 06536, USA.); Rudenko, Gloria (Sir Alexander Fleming Building, Dept. of Life Sciences, Imperial College London, U.K.)

Trypanosoma brucei uses antigenic variation of its Variant Surface Glycoprotein (VSG) coat to evade the host immune system. For antigenic variation to be effective, African trypanosomes use monoallelic exclusion to express a single VSG from one of ~15 bloodstream form expression sites (ESs). The active VSG ES is transcribed by RNA polymerase I (Pol I) in a nuclear compartment called the Expression Site Body (ESB). The ESB is believed to create a sub-nuclear environment that is critical for the regulation of monoallelic VSG expression. However, only a single protein to date, VEX1, has been shown to localise in close proximity to the active ES. We have now identified four novel proteins that form discrete nuclear foci that associate with the ESB, greatly expanding our knowledge of the protein factors interacting with the active ES. These include a homolog of the Cajal body protein NUFIP as well as three hypothetical proteins which we refer to as expression site associated proteins (EXAPs). We find by microscopy that EXAP1 always co-localises with VEX1, whilst TbNUFIP and EXAP2 co-localise in a separate nuclear body that is closely associated with the ESB. Interestingly, TbNUFIP, EXAP2 and EXAP3 have previously been shown to interact with CRK9, a protein essential for SL-RNA maturation. By directly inactivating the U2 snRNA using Morpholinos, we find that transcriptional processivity at the active ES and integrity of the ESB are rapidly lost upon splicing inhibition. We propose that the active ES recruits distinct nuclear bodies to facilitate its high demand for splicing and for the regulation of monoallelic exclusion.

1B A chromatin-associated VEX/CAF-1 complex drives VSG single gene choice and epigenetic inheritance in Trypanosoma brucei

Faria, Joana Glover, Lucy; Hutchinson, Sebastian (Institut Pasteur Paris); Boehm, Cordula; C. Field, Mark; Horn, David (Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee)

The largest gene families in eukaryotes are subject to allelic exclusion, but mechanisms underpinning single allele choice and inheritance remain mysterious. In African trypanosomes, association of one telomeric variant surface glycoprotein (VSG) gene with an RNA-polymerase-I (pol-I) transcription factory known as the expression-site body (ESB) facilitates singular VSG expression and antigenic variation. Having identified an ESB-associated factor, VSG exclusion 1 (VEX1), that mediates homology-dependent silencing, we sought VEX1-interactors through cryomilling and immunoprecipitation coupled with quantitative proteomics. We uncovered a ~1 megadalton complex where VEX1 is chromatin-associated and binds VEX2, an ortholog of nonsense-mediated-decay RNA-helicase UPF1, and chromatin-assembly-factor-1 (CAF-1), a conserved heterotrimeric replication-associated histone chaperone. VEX1 and VEX2 assemble in a transcription and splicing-dependent manner at the ESB-associated compartment, with VEX2-dependent VEX1 recruitment. CAF-1 binds and specifically maintains VEX1 compartmentalization following DNA replication. Following VEX2 or VEX1/VEX2 double knockdown, transcriptomic and proteomic analyses revealed that VSG transcripts and VSG coats become highly heterogeneous; indeed the 1,000-fold differential between active and silent VSGs expression is almost completely ablated. Moreover, following VEX2 knockdown, both the nucleolar and ESB reservoirs of pol-I decline. Thus, in trypanosomes, the VEX-complex underpins VSG-ES crosstalk and exclusion, while CAF-1 dependent VEX1 reloading provides allele-specific epigenetic inheritance; collectively sustaining a highly effective and paradigmatic host immune evasion strategy. Notably, UPF1 and CAF-1 have also been implicated in exclusion and inheritance functions in mammals. We suggest a model whereby VSG-ES transcription drives competition for a limited pool of the VEX-complex. Once associated with chromatin, the complex transmits a homology-dependent silencing signal, excluding other VSG-ESs and ultimately allowing only one VSG-ES to recruit sufficient pol-I and to form an ESB.

1C m6A methylation of the polyA-tail regulates VSG transcript stability in trypanosomes

Viegas, Idalio Rodrigues, João; Macedo, Juan (Instituto de Medicina Molecular); Aresta Branco, Francisco (Division of Immune Diversity, German Cancer Research Center, Heidelberg, Germany.); Jaffrey, Samie (Department of Pharmacology, Weill Cornell Medicine, Cornell University); Figueiredo, Luisa (Instituto de Medicina Molecular)

Trypanosoma brucei is covered by a dense coat of variant surface glycoproteins (VSG). The VSG transcript is very abundant, stable and has a half-life of around 4.5hr. The molecular basis for the stability of VSG mRNA is unknown. In other eukaryotes, N6-methyladenosine (m6A) has recently emerged as an RNA modification involved in post-transcriptional gene regulation. In this work, we hypothesized that m6A could be involved in stabilizing VSG mRNA. Using tandem-mass spectrometry, we first confirmed the presence of m6A in mRNA harvested from bloodstream and procyclic forms of trypanosomes, representing around 0,06% of adenosines, similar to other eukaryotes. Immunoblotting analysis revealed that m6A is enriched in the VSG transcript. RNAseH mapping showed that, unlike any other eukaryote, m6A is present in the polyA tail. To test the function of m6A in the polyA tail of VSG mRNA, we stopped transcription with actinomycin D and followed the dynamics of VSG mRNA decay, m6A removal and deadenylation of the polyA tail (the limiting step in transcript degradation). We observed that m6A removal precedes deadenylation of the polyA tail and VSG mRNA degradation. The same order of events was also observed during parasite differentiation to procyclic forms, suggesting that the previously described active degradation of VSG mRNA that happens during this process is also preceded by m6A removal from the polyA tail of VSG mRNA. Our data shows that VSG mRNA destabilization is preceded by m6A demethylation, suggesting that the high stability of VSG mRNA in trypanosomes is due to m6A in the polyA tail. This work identifies RNA modifications as a new mechanism to control the regulation of protein-coding genes in African trypanosomes

1D Trypanosoma brucei RAP1 has an RNA binding activity that is essential for VSG monoallelic expression

Gaurav, Amit K. Afrin, Marjia (Cleveland State University); Yang, Xian; Pan, Xuehua (The Hong Kong Polytechnic University); Sandhu, Ranjodh (University of California, Davis); Zhao, Yanxiang (The Hong Kong Polytechnic University); Li, Bibo (Cleveland State University)

Trypanosoma brucei causes African sleeping sickness in humans and regularly switches its major surface antigen, VSG, to evade the host immune response. VSGs are monoallelically expressed exclusively from expression sites located immediately upstream of telomeres, which are nucleoprotein complexes at chromosome ends. We have found that telomere proteins play important roles in VSG silencing and affect VSG switching. Specifically, T. brucei RAP1, a telomere protein, is essential for VSG silencing, suppresses the telomeric transcript (TERRA) and telomeric R-loop levels, and suppresses DNA recombination-mediated VSG switching events. However, the mechanism of TbRAP1-mediated telomeric silencing and VSG silencing is still poorly understood. Among RAP1 homologues, Saccharomyces cerevisiae RAP1 has both transcription activation and repression functions in a context-dependent manner, which depend on scRAP1's DNA binding activity that is mediated by its central Myb and Myb like domains. Similarly, scRAP1-mediated telomeric silencing is an epigenetic effect also dependent on its telomere DNA binding activity and its interaction with other silencers. To our great surprise, we now find that TbRAP1 has an RNA binding activity in vitro and interacts with the active VSG mRNA in vivo. In addition, structural analysis indicates that TbRAP1 has a putative RNA-binding domain. Most importantly, mutations that abolish TbRAP1's RNA binding activity lead to strong VSG derepression and subsequent growth defect, indicating that TbRAP1's RNA binding activity is essential for VSG monoallelic expression and cell viability. The RNA-binding activity is completely novel among all known RAP1 homologues, indicating more drastic evolution of telomere proteins than we have originally understood. Importantly, the discovery of TbRAP1's RNA-binding activity suggests an unexpected and novel mechanism underlies TbRAP1's role in silencing subtelomeric genes.

1E Active expression site ESAGs are not essential in vitro, but their presence alters VSG switching

Chamberlain, James Gadelha, Catarina (University of Nottingham, Nottingham, United Kingdom)

In bloodstream-form Trypanosoma brucei, the active VSG is transcribed from 1 of ~15 subtelomeric Bloodstream Expression Sites (BESs). These BESs also contain genes from up to 13 distinct families of co-transcribed Expression Site Associated Genes (ESAGs). Most ESAGs encode proteins that localise to the cell surface, and some are involved in the uptake of host macromolecules and modulation of the immune system. However, the function of most ESAGs remains unclear, as does their relationship to the wider families of Genes Related to ESAGs (GRESAGs), which are present elsewhere in the genome.

To disentangle the functions of (GR)ESAG families, we designed a system to test for the functional contribution of BES-encoded ESAGs versus their families as a whole. Surprisingly, this showed that although some (GR)ESAG families are necessary for normal cell growth, the actively-expressed ESAG is dispensable in all cases. Furthermore, using site-specific recombination we engineered a set of truncated BESs – including full removal of all ESAGs. All truncations are viable in vitro and only removal of the genes encoding the heterodimeric transferrin receptor (with/without all other ESAGs) has a mild fitness cost. All truncations produce single, active Expression Site Bodies, but removal of different regions results in 100-fold modulation of the rate of switching from the active VSG. Together these findings show that association with the active BES is unnecessary for (GR)ESAG family function in culture, and strongly suggest that GRESAG members alone (or leaky transcription of ESAGs from silent BESs) are functionally sufficient for any fitness conferred by the proteins. However, the presence of these genes in the active BESs has a significant impact on antigenic variation.

1F Developmental competence and antigen switch frequency can be uncoupled in Trypanosoma brucei

McWilliam, Kirsty Mugnier, Monica (Johns Hopkins University); Morrison, Liam; Matthews, Keith (University of Edinburgh)

African trypanosomes use an extreme form of antigenic variation to evade host immunity. This involves the switching of expressed variant surface glycoproteins, antigen exchange being a stochastic and parasite intrinsic process. Parasite development in the mammalian host is another feature of the infection dynamic, with trypanosomes undergoing quorum sensing-dependent differentiation between proliferative slender form and arrested, transmissible, stumpy forms within each parasitaemic wave. Longstanding experimental studies have suggested that the frequency of antigenic variation and transmissibility may be linked, antigen switching being higher in fly-transmissible, developmentally competent, parasites and lower in serially passaged lines. Here, we have directly tested this tenet of the infection dynamic by, firstly, generating lines that inducibly lose developmental capacity through RNAi mediated silencing of components of the stumpy induction signalling cascade ('inducible monomorphs'). Secondly, we have derived de novo lines that have lost the capacity for stumpy formation by serial passage ('selected monomorphs') and analysed their antigenic variation in comparison to isogenic pre-selected populations. Analysis of both inducible and selected monomorphs by in vitro flow-cytometry based VSG switch assays and VSGseq has established that antigen switch frequency does not change regardless of the method used to prevent parasite development. We conclude that changes in antigen switch frequency and developmental capacity can be uncoupled, these independently selected traits being important contributors to the parasite infection dynamic.

TT1G TURNOVER OF VSG IN TRYPANOSOMA BRUCEI

Garrison, Paige Umaer, Khan; Bush, Peter; Bangs, Jay (University at Buffalo)

African trypanosomes (Trypanosoma brucei) utilize the GPI-anchored virulence factor, Variant Surface Glycoprotein (VSG), to evade the immune system and maintain persistent infection in the mammalian host. VSG is very stable; turnover is thought to be mediated by slow cleavage of the GPI anchor by endogenous GPI-specific phospholipase C (GPI-PLC) leading to release from the cell surface. More recently, Szempruch et al. (Cell, 2016, pp246–257) have shown that bloodstream form trypanosomes release nanotubes that bud to form free VSG+ extracellular vesicles (EVs), suggesting an alternate mode of VSG turnover. Here we evaluate the relative contributions GPI-PLC cleavage and EV formation to VSG turnover in wild type (WT) and GPI-PLC Null cell lines. VSG turnover slows significantly (t1/2 ~36 hrs) in Null cells compared to WT (t1/2 ~26 hrs). Concomitantly, VSG release into the media is reduced in Null cells. However, floatation experiments indicate that the majority of VSG released from WT cells is membrane-associated, while a minor non-floatable portion is reactive with anti-CRD, a specific reagent for hydrolyzed GPIs. In Null cells all released VSG is membrane-associated and CRD-negative. GPI-PLC knockout has no effect on normal biosynthetic secretory trafficking, but does reduce specific uptake of transferrin as reported previously (Subramanya et al. Biochem. J., 417:685, 2009). Additional studies using RNAi to inhibit VSG synthesis indicate that cells do not alter the normal rate of turnover to conserve lateral cell surface VSG density. Overall our data suggest that the principal mode of VSG turnover in BSF cells is by EV shedding, and that trypanosomes do not regulate VSG turnover according to VSG protein abundance.

TT1H How DNA breaks contribute to mosaic VSG formation in Trypanosoma brucei?

Romero-Meza, Gabriela Mugnier, Monica

Unraveling the mysteries of antigenic variation in Trypanosoma brucei has been a challenge for many years. T. brucei uses a set of Variant Surface Glycoprotein (VSG) encoding genes to outsmart the mammalian immune system. The parasite membrane is covered by a dense coat of VSG that is regularly switched. In the T. brucei genome, there are ~2,000 VSGs, of which 80% are incomplete genes or pseudogenes. These genes can be used in gene conversion events to form mosaic VSGs through molecular mechanisms that are poorly understood. Experiments have shown that the induction of an exogenous DNA double strand break (DSB) near the actively expressed VSG can induce VSG switching, but the role of DSBs in the formation of mosaic VSGs has not been deeply explored. We developed an inducible CRISPR/Cas9 system to generate DSBs in active and silent VSGs to investigate the contribution of DSBs to VSG switching and mosaic formation. We validated the system by transfecting to Cas9-expressing cells with both a guide RNA targeting a single copy gene and a repair template containing GFP. We observed ~0.2% of parasites expressing GFP, confirming that the system can efficiently generate DNA breaks. We next plan to examine the effect of targeting DNA breaks to various locations throughout the genometo determine whether these breaks can lead to the formation of mosaic VSGs. To evaluate the outcomes after induction of a break, we are using anchored multiplex PCR sequencing, a targeted sequencing approach that is capable of detecting gene fusions, insertions, deletions and point mutations. Using this method, we will not only quantitatively monitor the formation of mosaic VSGs after the induction of a DSB but also identify mosaic VSG donor sequences.

SESSION II:
Biochemistry/Metabolism
Ken Stuart, Chair.
Sunday 8:45 am
04/28/19

2A Glycerol, a new key player in the central carbon metabolism of African trypanosomes

Bringaud, Frédéric MFP (CNRS UMR-5234), University of Bordeaux, Bordeaux, France

African trypanosomes, like all other microorganisms, consume and metabolize carbon sources available in their immediate environment to increase their biomass and spread. In the glucose-rich fluids of the mammalian hosts, the bloodstream forms (BSF) of *Trypanosoma brucei* use glucose for their growth. In contrast, the procyclic forms (PCF) that propagate in the glucose-free midgut of the insect vector depend on proline, abundant in the fly. However, *in vitro*-grown PCF and BSF trypanosomes can replace their preferred carbon source with glycerol, which raises questions about the role of glycerol in the development and growth of trypanosomes in their insect and mammal hosts. This presentation will describe the peculiarities of glycerol metabolism in trypanosomes and examine their potential value for *in vivo* development of the parasites.

2B The Trypanosoma UDP-glucose pyrophosphorylase is active in the cytosol and glycosomes and is imported into the organelle by piggybacking

Villafraz, Oriana; Baudouin, Hélène; Mazet, Muriel (MFP (CNRS UMR-5234), University of Bordeaux, Bordeaux, France); Kulyk, Hanna; Portais, Jean-Charles (LISBP (CNRS, INRA, INSA), University of Toulouse, Toulouse, France)

Many glycosomal proteins from Trypanosoma brucei do not contain the standard peroxisomal targeting signals (PTS1 and PTS2). Theoretically, glycosomal import of PTS-lacking proteins can occur by piggybacking on a different PTS-containing protein, although it has never been observed for trypanosomatids so date. The UDP-glucose pyrophosphorylase (UGP), an enzyme involved in the synthesis of nucleotide sugars, is localized both in the cytosol and in the glycosomes of the procyclic trypanosomes, despite the absence of any known PTS signal. We showed that UGP is imported into the glycosomes by piggybacking on the glycosomal PTS1-containing PEPCK and identified the residues involved in the PEPCK-UGP interaction. Furthermore, we showed that the pathway involving UGP is functional in the glycosomes in addition to the canonical cytosolic pathway. Indeed, the lethality of RNAiUGP mutant cell lines was rescued by expressing a recoded UGP only in the organelle in the RNAiUGP/EXPrUGP-GPDH cell line. This conclusion was supported by targeted metabolomic analyses (IC-HRMS) showing that the product of the UGP reaction (UDP-glucose) is not longer detectable in the RNAiUGP mutant, while it is still produced in cells expressing UGP exclusively in the cytosol (Δpepck) or in the glycosomes (RNAiUGP/EXPrUGP-GPDH). Trypanosomatids are the only known organisms to have selected peroxisomal (glycosomal) sugar nucleotide biosynthetic pathways in addition to the canonical cytosolic ones. This raises questions about the role of this glycosomal pathways and how sugar nucleotides are exchanged between the glycosomal and cytosolic compartments before reaching the endoplasmic reticulum where it is required to feed biosynthesis of glycoconjugates.

2C Responses to reduced glucose transport in Trypanosoma brucei: gluconeogenesis and signaling

Kovarova, Julie Hutchinson, Sebastian; Horn, David (University of Dundee)

African trypanosomes express distinct hexose/glucose transporters in the bloodstream (THT1) and in the insect (THT2). We have explored responses to glucose transporter depletion in each life-cycle stage. Bloodstream-form T. brucei were thought to be completely dependent upon glucose for ATP production. However, we found that glucose transporters are dispensable when glycerol is available and that glycerol replaces glucose as a carbon and ATP source under these conditions. Metabolomic analyses with 13C-labelled glycerol revealed utilisation for gluconeogenesis and for ATP production, also in the pentose phosphate pathway and surface glycoprotein synthesis. Hence, contrary to prior thinking, gluconeogenesis does operate in bloodstream form trypanosomes; this pathway may be important for growth in adipose tissue. Consistent with gluconeogenic capacity, we detected robust fructose-1,6-bisphosphatase activity. Surprisingly, however, the activity is still present after CRISPR-Cas9 based deletion of the annotated fructose-1,6-bisphosphatase gene (Tb927.9.8720). We are currently determining whether sedoheptulose-1,7-bisphosphatase (Tb927.2.5800) provides this activity. Insect-stage T. brucei express the THT2 transporter. This transporter was the top hit in an RNAi screen in insect-stage cells for VSG promoter repression factors/pathways; other hits included kinases and a phosphatase. The THT2 gene was validated and this putative glucose-responsive signalling pathway will be explored.

2D Trypanosoma cruzi intracellular amastigote glutamine metabolism and its impact on susceptibility to ergosterol biosynthesis inhibitors

Dumoulin, Peter Burleigh, Barbara (Harvard T.H. Chan School of Public Health)

Chagas disease is the clinical manifestation of infection with the kinetoplastid parasite Trypanosoma cruzi. In its mammalian host, T. cruzi only replicates intracellularly as an amastigote. Persistent tissue parasitism is the central determinant of disease and therefore drug discovery/development efforts are aimed at eliminating intracellular amastigotes. A class of antifungal azole derivatives (ergosterol biosynthesis inhibitors – EBIs) performed well in pre-clinical assays, yet in clinical trials, these compounds suppressed but failed to clear infection. The nature of the persisting parasite population during azole treatment remains unknown. One hypothesis is that surviving parasites are in a metabolically protected niche and are refractory to EBIs. Consistent with this possibility, we have found that restriction of exogenous glutamine during intracellular T. cruzi amastigote replication in cell culture, protects the parasites from the cytotoxic effects of EBIs. Supplementation with α-ketoglutarate, a metabolic product downstream of glutamine, is sufficient to re-sensitize glutamine restricted parasites to EBIs. Since α-ketoglutarate can be generated by glutamate dehydrogenase (GDH) enzymes from glutamate derived from glutamine, we used CRISPR/Cas9 to generate functional knockouts of two distinct GDH genes in T. cruzi (1) an NADP-dependent GDH with predicted cytosolic localization (cGDH) and (2) a mitochondrial NAD-dependent GDH (mGDH). Both mutant lines were infective for mammalian cells but differed greatly in their sensitivity to EBIs. The cGDH mutant amastigotes were refractory to ketoconazole, similar to the protection observed with wild-type parasites under conditions of glutamine restriction, whereas the mGDH mutant parasites remained sensitive. Therefore, our findings implicate cGDH and the cytosolic pathway of reductive carboxylation of glutamine, that directly links glutamine catabolism with sterol synthesis, in the susceptibility of T. cruzi amastigotes to EBIs. Metabolomics experiments are underway to identify the biochemical processes that modulate the sensitivity of T. cruzi amastigotes to EBIs. These data underscore the potential for the immediate host metabolic environment to modulate the efficacy of anti-trypanosomal compounds that target parasite metabolism.

2E A foot in the door: Understanding nutrient stress tolerance and adaptive gene regulation in Leishmania donovani through the lens of a purine-responsive transporter

Licon, Haley Soysa, Radika (University of Washington); Landfear, Scott; Yates, Phillip (Oregon Health and Science University)

Responding to changes in the host is critical for survival of the kinetoplastid parasite, Leishmania. Aspects of adaptation are likely unique, as the polycistronic nature of transcription precludes gene regulation at the level of mRNA synthesis in kinetoplastids. As Leishmania are auxotrophic for purines, we have used purine starvation as a paradigm to study mechanisms of nutrient stress tolerance in Leishmania donovani. We previously showed that the L. donovani proteome is dramatically remodeled under purine stress, greatly enhancing our understanding of what metabolic changes are involved in withstanding purine deprivation. However, how these changes are established remained ill-defined.

Here, we examine the leishmanial purine stress response through the lens of a representative purine-responsive gene. The purine nucleobase transporter, LdNT3, represents one of the most substantially upregulated proteins in purine-starved L. donovani. We have published that LdNT3 upregulation is mediated (at least, in part) at the levels of mRNA stability and translation. Now we show that LdNT3 protein stability is not altered under purine stress, establishing the dominance of mRNA-level and translational control points in purine-responsive LdNT3 expression. We have identified a 33-nucleotide stem-loop in the 3' UTR that serves to repress LdNT3 expression when extracellular purines are abundant. Notably, we demonstrate that this element is necessary for purine-responsive regulation, and is sufficient to confer regulation outside of its endogenous context. Together, these observations mark an important first step toward understanding purine stress tolerance in Leishmania. Future efforts will seek to identify factors that associate with this stem-loop, and ultimately, map the network of RNA-protein and protein-protein interactions that govern this response on a global scale.

2F Remodeling the mitochondrial proteome and metabolic networks of Trypanosoma brucei during in vitro insect stage differentiation

Zikova, Alena Dolezelova, Eva (Biology Centre); Levin, Michal; Dejung, Mario (Institute of Molecular Biology); Kunzova, Michaela; Panicucci, Brian (Biology Centre); Janzen, Christian; Butter, Falk (University Wuerzburg)

Throughout the Trypanosoma brucei life cycle, the mitochondrion undergoes extensive structural and metabolic rewiring as it transforms from an ATP-producing organelle in the procyclic form to ATP-consuming in the bloodstream form. Complex mitochondrial remodeling can now be examined in vitro during procyclic differentiation induced by overexpressing a single RNA binding protein, RBP6. Our transcriptome and cellular proteome data indicate that the most significant changes occur within 2 days of RBP6 induction when 90% of the culture has differentiated into epimastigotes. Thereafter, only minor additional adaptations were observed as ~50% of the culture further differentiated into metacyclic trypomastigotes by day 6. The omics data are consistent with our bioenergetic results that demonstrate i) an immediate redirection of electron flow from the OXPHOS pathway to an alternative oxidase, which leads to decreased total ATP levels; ii) the progressive reduction of respiratory complexes III and IV; and iii) the increased abundance and activity of certain TCA cycle enzymes and complex II. Increased complex II activity can lead to reactive oxygen species (ROS) production and indeed higher levels of mitochondrial ROS were measured. Furthermore, the exogenous expression of catalase, a ROS scavenger, severely limits the effect of RBP6 induction on procyclic differentiation, suggesting that ROS are acting as a retrograde signal. Our findings provide insights into the emerging concept that mitochondria act as signaling organelles through the release of TCA cycle derived metabolites and ROS to drive cellular differentiation.

2G Deciphering the mitochondrial quality control mechanism in Trypanosoma brucei

Dewar, Caroline Oeljeklaus, Silke; Suppanz, Ida; Peikert, Christian (University of Freiburg); Mani, Jan (University of Bern); Warscheid, Bettina (University of Freiburg); Schneider, André (University of Bern)

Mitochondrial quality control is the network of pathways by which eukaryotic cells monitor and maintain the function of their mitochondria. Trypanosoma brucei has only a large single mitochondrion, which prevents the elimination of dysfunctional mitochondria as in some other organisms. When this essential mitochondrion is not functioning correctly, for example when mitochondrial protein import is defective, cell viability suffers. The processes by which this parasite counteracts declining mitochondrial functionality are therefore of great interest.

95% of mitochondrial proteins in T. brucei are encoded in the nuclear DNA. The multisubunit ATOM complex is the mediator of protein import through the mitochondrial outer membrane in trypanosomes (Pusnik et al., 2011, Mani et al., 2015). Using a depletomics approach to target this complex, we defined the T. brucei mitochondrial importome as being the proteins which decreased in level when ATOM40, the central import pore, was knocked down (Peikert et al., 2017). Interestingly, adding a proteasome inhibitor causes a restoration in the levels of mitochondrial import substrates. The proteasome is therefore involved in the cytosolic degradation of non-imported mitochondrial substrates in the presence of an import defect.

Here, using a variety of proteomic and biochemical approaches, we show that the proteasome and putative components of a ubiquitin-driven pathway are recruited to the outer mitochondrial membrane upon the induction of an import defect. To facilitate exposure of membrane proteins to the cytosolic proteasome, AAA-ATPase homologs p97 and MSP1 play a synergistic role in the extraction of membrane proteins from the outer mitochondrial membrane. We have verified the role of recruited proteins in the quality control pathway, and are currently investigating their interaction partners. We are also examining factors that govern susceptibility of substrates to this pathway.

TT2H Targeting the ubiquitin system of Leishmania Mexicana

Burge, Rebecca Damianou, Andreas; Catta-Preta, Carolina (The University of York); Rodenko, Boris (UbiQ); Mottram, Jeremy C. (The University of York)

Ubiquitination is a widespread post-translational modification with diverse roles including in proteasomal degradation, endocytic trafficking and DNA repair. The key enzymes of ubiquitination, which act in a sequential manner to activate, carry and transfer ubiquitin onto a substrate protein respectively, were identified encoded in the L. mexicana genome: 2 E1 ubiquitin-activating enzymes (E1s), 15 E2 ubiquitin-conjugating enzymes (E2s) and 80 E3 ubiquitin ligases (E3s). 20 deubiquitinating enzymes (DUBs), which remove these modifications, were also identified. Previously, trypanosomal-specific proteasomal inhibition has been shown to reduce parasite burden in animal models of both visceral and cutaneous leishmaniasis. Therefore ubiquitination enzymes, which operate upstream and are more substrate-selective than the proteasome, may be a good source of new, anti-leishmanial drug targets. Subsequent generation of a null mutant E1, E2, HECT/RBR E3 and DUB library using a CRISPR/Cas9-based approach revealed around 18% of these genes could be essential in promastigotes. Further library analysis using a pooled bar-seq strategy revealed numerous life cycle defects in multiple cell lines. In particular, the loss of either of two E2s, UCE2 and UCE5, led to similarly severe loss-of-function defects in the amastigote stage. Additionally, UCE2 and UCE5, which are closely related to the heterodimer-forming human E2s UBC13 and UEV1A, were shown to form a stable interaction in vitro. A possible role for L. mexicana DUB2 in antagonising the ubiquitination activity of UCE2/UCE5 will also be described. These results highlight the importance of the ubiquitination system for L. mexicana differentiation, survival and virulence and provide a foundation for further work into the roles of these enzymes in Leishmania biology.

TT2I Identifying highly divergent glycosyltransferases in the African Trypanosome

Duncan, Samuel Damerow, Manuela; Ferguson, Michael (The University of Dundee)

Trypanosoma brucei expresses a diverse repertoire of N-glycans, ranging from oligomannose and paucimannose structures to exceptionally large complex N-glycans, as well as glycosylphosphatidylinositol (GPI) anchored proteins. We estimate that protein glycosylation in this parasite requires at least 38 distinct glycosyltransferases (GTs), only a few of which can be predicted by bioinformatics. A family of 21 putative trypanosome GTs has been identified that share a single β1-3 transferase ancestor but catalyse a diverse array of glycosidic linkages. Inhibition of such highly divergent GTs is therefore a promising therapeutic avenue, yet 17 of these putative TbGTs require characterisation. In this study we have assessed the function of GT10. Conditional gene deletion reveals that protein expression is not essential in vitro, but leads to impaired elaboration of N-glycans in BSF cells as assessed by lectin blotting. Using antibodies specific to lysosomal p67 peptide and carbohydrate epitopes we demonstrate that glycosylation of this protein is severely disrupted in GT10 deficient cells, leading to impaired p67 processing and increased resistance to Suramin at a level similar to p67 RNAi. Interestingly, GT10 has a dual function as differentiation of BSF null mutants to PCF in vitro reveals a reduction in the molecular weight of EP and GPEET procyclin, indicative of reduced GPI-anchor glycosylation. This phenotype will enable the functional characterisation of GT10 by permethylation of GPI anchored procyclins and subsequent MS3 analysis to identify the sugar linkage absent in GT10 null cells.

SESSION III:
Cell Biology
Kimberly Paul
Sunday 2:00 pm
04/28/19

3A Cryo electron tomography with Volta phase plate reveals novel structural foundations of axoneme motility in the pathogen Trypanosoma brucei

Imhof, Simon Zhang, Jiayan (Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California, USA); Wang, Hui (California NanoSystems Institute, UCLA); Huy Bui, Khanh (Department of Anatomy and Cell Biology, McGill University, Montreal, CA); Nguyen, Hoangkim (Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California, USA); Atanosov, Ivo; Hui, Wong; Zhou, Hong (California NanoSystems Institute, UCLA); Hill, Kent (Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California, USA)

A microtubule axoneme is the foundation for all eukaryotic flagella and cilia. Recent cryoEM studies have transformed our understanding of axoneme structure, providing insight into mechanisms of axoneme assembly and function. To date however, no high resolution structures are available for organismsin the Excavata phylogenetic lineage, which includes several pathogens of medical and economic importance. Here we employ cryo electron tomography (cryoET) with Volta phase plate, energy filtering and direct electron detection to determine the 3D structure of the axonemal repeat from Trypanosoma brucei. We examined axonemes from two different developmental stages, bloodstream and procyclic culture form, as well as a mutant lacking the DRC11 subunit of the nexin dynein regulatory complex (NDRC). Sub-tomogram averaging of the 96-nm repeat yields a resolution of 21 Å overall and 11 Å for the doublet microtubules. We discovered several lineage-specific structures, including extensive and novel inter-doublet linkages and an expanded cohort of microtubule inner proteins (MIPs) compared to other organisms. We also establish that DRC11/CMF22 is part of the NDRC proximal lobe that binds the adjacent doublet microtubule. Lineage-specific elaboration of axoneme structure in T. brucei may reflect adaptations to support unique motility needs in diverse host environments.

3B The Leishmania flagellum attachment zone is critical for flagellar pocket shape and development in the sand fly and pathogenicity in the host

Sunter, Jack Yanase, Ryuji (University of Hyogo); Wang, Ziyin (University of Oxford); Catta-Preta, Carolina (University of York); Moreira-Leite, Flavia (University of Oxford); Myskova, Jitka; Pruzinova, Katerina; Volf, Petr (Charles University); Mottram, Jeremy (University of York); Gull, Keith (University of Oxford)

In considering functions important for pathogenicity, it is evident that the shape of a single-celled parasite will have been honed by the evolutionary arms race between host and parasite. Leishmania parasites have a distinct cellular architecture depending on location in the host or vector and specific pathogenicity functions. An invagination of the cell body membrane at the base of the flagellum, the flagellar pocket, is an iconic kinetoplastid feature, and is central to processes that are critical for Leishmania pathogenicity. The Leishmania flagellar pocket has a bulbous region posterior to the flagellar pocket collar, and a distal neck region where the flagellar pocket membrane surrounds the flagellum more closely. The flagellum is attached to one side of the flagellar pocket neck by the short flagellum attachment zone (FAZ). We addressed whether targeting the FAZ affects flagellar pocket shape and its function as a platform for host-parasite interactions. Deletion of the FAZ protein FAZ5 clearly altered flagellar pocket architecture and had a modest effect on endocytosis but did not compromise cell proliferation in culture. However, FAZ5 deletion had a dramatic impact in vivo: mutants were unable to develop late stage infections in sand flies and parasite burdens in mice were reduced by >97%. Our work demonstrates the importance of the FAZ for flagellar pocket architecture and function, and that deletion of a single FAZ protein can have a large impact on parasite development and pathogenicity.

3C Structural studies of the interaction between FPC3 and FPC4, two components of the flagellar pocket collar in Trypanosoma brucei

Pivovarova, Yulia Majneri, Paul; Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria); Robinson, Derrick R.; Bonhivers, Melanie (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)

Trypanosoma brucei is a unicellular parasite transmitted by tsetse flies. It is the causative agent of sleeping sickness that threatens more than 60 million people in sub-Saharan countries. T. brucei has a unique flagellar pocket at the base of its flagellum, which is the sole site for all endo- and exocytic activities in the cell, and thus an attractive therapeutic target. At the neck of the flagellar pocket is a belt-like cytoskeletal structure called the flagellar pocket collar (FPC). We have previously characterized the structure and assembly of a component of the FPC, BILBO1, which serves as an anchor and scaffold for the docking of other FPC components to maintain the structure and function of FPC. FPC3 is one of the few newly identified FPC proteins, sharing 30% sequence identity with the N-terminal domain (NTD) of BILBO1. Yeast two-hybrid and biochemical assays demonstrated that FPC3-NTD interacts with the C-terminal domain (CTD) of another FPC protein and BILBO1 partner, FPC4.

We have recently solved a 1.6-Å resolution crystal structure of FPC3-NTD in complex with FPC4-CTD, which is the first complex structure of any two FPC components. The structure revealed that FPC3-NTD shares a similar fold to that of BILBO1-NTD. Six residues of FPC4-CTD are tightly clamed into a conserved hydrophobic pocket in FPC3-NTD. Further structure-based mutagenesis studies revealed residues on both proteins that are most critical for their interaction. Our structural work provides the first clear view of the interaction between two FPC proteins, which may be used in the future for designing inhibitors for therapeutic intervention, such as small molecules or modified polypeptides to disturb the binary interaction in trypanosomes.

3D Characterization of TAP110, a mitochondrial protein associated with the TAC, in Trypanosoma brucei

Amodeo, Simona Schimanski, Bernd; Ochsenreiter, Torsten (University of Bern, Switzerland)

Mitochondrial genome inheritance has been extensively studied in only a handful of model organisms, all belonging to the Opistokonta eukaryotic super group. To understand mitochondrial evolution in more detail, it is important to include organisms of other super groups such as Excavata (e.g. T. brucei) in our studies.

To assure correct inheritance of the replicated kDNA to the two daughter cells in T. brucei, it is anchored to the flagellum that drives kDNA segregation during cell division.The structure connecting the kDNA to the basal body has been described as the tripartite attachment complex (TAC). Several components of that TAC structure and how they assemble has been described in the last few years. It remains elusive how the TAC, inside the mitochondrion, is able to connect to the kDNA.

Here, we present data on the TAC associated protein 110 (TAP110). TAP110 is a 110 kDa protein that shows sequence similarities to a histone and microtubule-binding protein. With super-resolution microscopy, we show that TAP110 co-localizes with TAC102, a TAC component of the unilateral filaments in the mitochondrion. Blue native and co-immunoprecipitation analyses suggest that TAP110 is an interaction partner of TAC102. We demonstrate that the TAC, but not the kDNA, is required for correct TAP110 localization. Depletion of TAC102 leads to loss of TAP110, suggesting that TAP110 is more proximal to the kDNA than TAC102. Interestingly, TAP110 can only be solubilized in dyskinetoplastic cells suggesting a direct interaction with the kDNA.

3E Processing and targeting of cathepsin L (TbCatL) to the lysosome in Trypanosoma brucei

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

Cathepsin L (TbCatL) is a lysosomal thiol protease in African trypanosomes. TbCatL is synthesized as two precursor forms (full-length P and smaller X of uncertain identity) with an N-terminal prodomain that mediates post-Golgi sorting, which is removed upon arrival in the lysosome to generate mature enzyme (M). Post-Golgi sorting of TbCatL to the lysosome in procyclic parasites is clathrin-mediated. We examine TbCatL trafficking in a novel system: truncated TbCatL reporter without the C-terminal domain (CTD; TbCatLΔ) ectopically expressed in an RNA interference (RNAi) cell line targeting the CTD/3′ untranslated region (UTR) of endogenous mRNA. TbCatLΔ is synthesized as P′/X′/M′ species, localizes to the lysosome, and rescues the lethal TbCatL RNAi phenotype. Inactive TbCatLΔ:C150A is only processed to M′ in the presence of endogenous TbCatL indicating trans-auto-catalytic activation. X′ is formed with active endoplasmic reticulum (ER)-retained TbCatLΔ:MDDL, but not with TbCatLΔ:C150A, indicating stochastic generation in the ER by cis-auto-cleavage within the prodomain of newly synthesized P′. TbCatL modeled on the known structure of human CatL suggests three solvent accessible features that could contain post-Golgi targeting signals: the N-terminus, an α-helix:turn junction (α1-T1), and a separate turn (T3). Mutation of E34/E35 in the N-terminus had no effect on trafficking. K47, G49, and K50 of the α1-T1 region had previously been implicated in trafficking of the T. cruzi orthologue [Huete-Perez et al. (1999) JBC 274:16249], but we found no such effect in T. brucei. However, the N79P80 dipeptide in T3 that is strictly conserved in all Kinetoplastida is a critical motif for lysosomal targeting. These findings show novel insights on the maturation of TbCatL, which is a critical virulence factor in mammalian infection.

TT3F Swim like your lifecycle depends on it: Investigating chemotaxis and navigation in Leishmania parasites

Walrad, Pegine B. Findlay, Rachel; Osman, Mohamed (York Biomedical Research Institute); Gadelha, Hermes (Department of Mathematics); Kaye, Paul (York Biomedical Research Institute); Rogers, Matthew (London School of Hygeine and Tropical Medicine); Wilson, Laurence (Biological Physical Sciences Institute, University of York)

Motility of Leishmania parasites is essential for survival and lifecycle progression. An oscillating flagellum pulls the promastigote through its changing environment and the parasite transforms to optimise its infection potential. During the host transition from sandfly vector to mammalian hosts, the egestion from the sandfly is highly enriched in metacyclic promastigotes due to the viscous promastigote secretory gel (PSG) creating a 'sieve'. Details of how the human-infective metacyclics are capable of swimming through this substance compared to other promastigote stages remains obscure, but is fundamentally linked to infectivity and transmission and is therefore the focus of our investigation. We use a novel imaging technology – digital inline holographic microscopy (DIHM) – to track promastigote swimming in three dimensions (3D). Tracking the swimming behavior of >10,000 cells, we find differences between the promastigote stages: procyclic promastigotes swim slower with a persistent 'corkscrew' motion and metacyclic forms swim faster with a striking run-and tumble motif similar to chemotactic bacteria (E. coli). Both lifecycle stages were placed in controlled chemical gradients, and their swimming behavior examined. While the procyclic swimming behavior did not change, the metacyclics undergo a chemokinetic shift and swim toward macrophages. In the presence of a visco-elastic fluid, both lifecycle stages' swimming speed slows but the helicity of the metacyclics specifically increases. As in the sandfly, the metacyclics swim more easily in a complex fluid, displaying higher levels of motility and faster swimming speeds in PSG. We quantitatively demonstrate that metacyclic cells are more capable of swimming through PSG and demonstrate chemotaxis in the presence of cells they infect.

TT3G The dual FAZ domain-localizing protein FAZ27 cooperates with FLAM3 and ClpGM6 to control morphology transitions in Trypanosoma brucei

AN, TAI ZHOU, QING; HU, HUIQING; LI, ZIYIN

Trypanosoma brucei undergoes life cycle form transitions from trypomastigotes to epimastigotes in the tsetse fly vector by re-positioning the kinetoplast, the flagellum, and flagellum-associated cytoskeletal structures towards the anterior portion of the cell. The molecular mechanism underlying this drastic morphology change remains poorly understood. Previous work identified the requirement of multiple flagellum attachment zone (FAZ) components and flagellar proteins, including the FAZ flagellum domain proteins FLAM3 and ClpGM6 and the flagellar tip-localizing orphan kinesin KIN-E, in controlling the morphology transition. Here we report the identification of a dual FAZ domain-localizing protein named FAZ27 and the characterization of its mechanistic role in controlling morphology transitions. FAZ27 localizes to both the FAZ flagellum domain and the FAZ filament domain, and BioID using FAZ27 as the bait identified proximal proteins from the FAZ flagellum domain, such as FLAM3 and ClpGM6, and the FAZ filament domain, such as FAZ10, FAZ19 and KMP-11. Knockdown of FAZ27 in the trypomastigote form of T. brucei causes a major change of cell morphology by producing epimastigote-like cells, suggesting a role for FAZ27 in controlling cell morphology transitions. Co-immunoprecipitation showed that FAZ27 interacts with FLAM3 and ClpGM6 in vivo, suggesting that they form stable protein complexes in the FAZ flagellum domain. Further, analyses of the functional interdependence among the three proteins revealed distinct roles in mediating protein localization and stability. While FAZ27 and FLAM3 are interdependent for subcellular localization, FAZ27 is required for maintaining ClpGM6 protein stability and ClpGM6 is required for FAZ27 localization but not stability. Together, FAZ27 is the first protein to be localized to dual domains of the FAZ, and it cooperates with FLAM3 and ClpGM6 to maintain trypomastigote cell morphology by controlling the assembly of the FAZ in both the extracellular FAZ flagellum domain and the intracellular FAZ filament domain.

TT3H TrypTag: Biological lessons and opportunities from a molecular atlas of trypanosomes

Dean, Samuel Billingdon, Karen (University of Oxford); Halliday, Clare (Oxford Brookes); Madden, Ross (University of Oxford); Carrington, Mark (University of Cambridge); Hertz-Fowler, Christiane (University of Liverpool); Vaughan, Sue (Oxford Brookes); Gull, Keith (University of Oxford); Sunter, Jack (Oxford Brookes); Wheeler, Richard (University of Oxford)

The core TrypTag project is now complete, providing localisation data on nearly every protein encoded by the T. brucei genome. We have generated >14,000 tagged cell lines and imaging data covering 91% of non-VSG genes encoded in the genome, meaning that T. brucei is now the second unicellular eukaryote ever (after yeast) to have such a resource. This dataset comprises >71,000 images of >4,400,000 cells.

Genome-wide localisation data provides unique ways to discover new biology. We have discovered where thousands of uncharacterised proteins are likely to function, generating understanding not possible from other approaches: For example, the proteins present in novel sub-domains of organelles – particularly in the endoplasmic reticulum (ER), cytoskeleton, mitochondrion, nucleus. There are 6 ER subdomains, including a flagellar pocket-associated domain, each with characteristic protein content, providing insight into trypanosome-specific adaptation. We have determined the relative complexity of each trypanosome organelle and found that the nucleus (~1200 proteins), mitochondrion (~1100 proteins) and flagellum (~800 proteins) are by far the most complex organelles, the basal body alone being constructed from >280 components. In comparison, only 50 proteins localised to the cortical cytoskeleton. This gives an unprecedented view into the evolution of parasite complexity. These are just two examples of the plethora of genome-wide analyses we have now completed.

The TrypTag atlas provides many opportunities for individual researchers to predict biology and develop testable hypotheses for the functions of the thousands of genes. This data is freely available on TrypTag.org and TriTrypDB.org.

TT3I High frame rate multi-colour and multi-focal plane microscopy to gain new insights into trypanosomatid parasite swimming

Wheeler, Richard J.

Leishmania and Trypanosoma brucei both undergo two major modes of swimming driven by different flagellar beats. One with a tip-to-base symmetrical waveform (the flagellar-type beat) to drive forward swimming and one with a base-to-top waveform which is, in Leishmania, asymmetrical (the ciliary-type beat) and drives cell turning. This motility is vital for progression through the life cycle.

Fluorescence from endogenously tagged proteins is powerful for modern cell biology questions but observing fluorescence in live beating flagella is extremely challenging. Trypanosomatid flagella beat tens of times per second, demanding camera frame rates upward of 200 frames per second, with flagellar contortions into three-dimensionality. I developed multi-focal plane and dual colour widefield fluorescence microscopy capable of analysing this motion using proteins endogenously tagged with fluorescent reporters. High frame rate multi-focal plane microscopy allowed the direct analysis of the three-dimensional configuration of beating flagella, including the use of SMP1::mNG fluorescence in Leishmania mexicana to reveal the degree to which beating deviates from planarity. High frame rate dual colour microscopy allowed observation of flagellum structures while swimming, including mNG::PFR RSP4/6::mCh and mNG::SPEF1 SMP1::mCh to show that the Leishmania ciliary beat has a preferred bend direction.

This ability to visualise multiple focal planes and multiple colours in live beating flagellum opens new opportunities to understand how Leishmania and related parasites control their swimming. These techniques are also transferrable to other flagellated/ciliated organisms.

TT3K UNRAVELLING THE ROLE OF PROTEIN KINASES IN LEISHMANIA DIFFERENTIATION

Baker, Nicola Catta-Preta, Carolina; Neish, Rachel; Powell, Ben; Ferreira, Eliza; Geoghegan, Vincent; Newling, Katherine; Pitchford, John; Mottram, Jeremy (University of York)

Leishmania encounter a multitude of environmental stresses during transmission between the extracellular promastigote stages in the sandfly and intracellular amastigote stages in mammalian macrophage phagolysosomes. Leishmania mexicana has 194 protein kinases and 11 atypical protein kinases, each thought to play an important role in environmental stress signalling, cell cycle control and differentiation via phosphorylation. 29 of these kinases are not found in trypanosomes and may have Leishmania-specific roles.

We have taken a kinome-wide approach to unravel the localisation and requirement of each protein kinase throughout the life cycle. CRISPR-Cas9 editing was used to both endogenously tag and delete each protein kinase gene individually in promastigotes. 159 null mutant lines were produced, each with an identifying barcode. Null mutants could not be produced for 43 kinases, which we therefore assume are required for promastigote survival. The non-essential null mutants were pooled and assessed for their ability to differentiate and to respond to specific stresses. Bar-seq was employed to identify the percentage representation of each mutant throughout the life cycle from promastigote to metacyclic and amastigote stage (axenic, macrophage and mouse infection). These were plotted as log trajectories and subjected to projection pursuit clustering in order to identify phenotypic groups. We identified 44 protein kinases required for the infective amastigote stage, 4 of which are required for in-vivo mouse infection only and 2 of which are unique to Leishmania.

Together the localisation and Bar-seq data provide a new wealth of information about many previously uncharacterised protein kinases in Leishmania and provide a basis to try and start deconvoluting kinase signalling pathways important for stress response, life cycle progression and infection.

SESSION IV:
Pathogenesis I
Luisa Figueiredo, Chair.
Monday 8:45 am
04/29/19

4A The life of trypanosomes in the tsetse fly
Roditi, Isabel

By 1896 David Bruce had shown that trypanosomes were transmitted by tsetse flies, but transmission was thought to be mechanical, and it took much longer before it was realised that the parasites underwent a considerable part of their developmental cycle in the insect host. Keith Vickerman aptly described this as a "journey fraught with hazards" as the parasites not only have to counter the fly's innate immune system, but also have to cross substantial physical barriers. Transmission of *Trypanosoma brucei* takes approximately three weeks and involves an ordered set of differentiation, proliferation and migration steps in several distinct tissues. While many parts of the cycle were correctly elucidated more than a hundred years ago, others were discovered only recently and there are still considerable, and surprising, gaps in our knowledge. On the positive side, technologies such as next generation sequencing and super resolution microscopy are delivering new insights and altering our perception of how trypanosomes interact with their host.

4B Chemotaxis in trypanosomes – it's all about the basics

Shaw, Sebastian Naguleswaran, Arunasalam; Roditi, Isabel (Institute of Cell Biology, Bern, Switzerland)

Trypanosoma brucei spp colonise a variety of tissues in their insect and mammalian hosts. At present, virtually nothing is known about how trypanosomes home into different tissues and how they overcome physical barriers. We have recently shown that trypanosomes lacking flagellar phosphodiesterase B1 are unable to traverse the peritrophic matrix that separates the tsetse midgut lumen from the ectoperitrophic space1. As a consequence, development is blocked and the parasites cannot complete the life cycle. One possibility is that the parasites require cAMP signalling to sense and move along chemotactic gradients. The group movement of trypanosomes in response to external stimuli (social motility) can be monitored on agarose plates. When early procyclic forms are inoculated onto the surface, they multiply until they reach a threshold number, at which point they start to form evenly spaced radial projections containing tens of thousands of cells. Radial projections from two communities on same plate are able to sense each other and reorient, suggesting that they detect a repellent. We now show that early procyclic forms react to pH by changing the direction of migration. Parasites are repelled by acids and attracted to alkaline solutions. The PDEB1 knockout does not show social motility1, nor does it respond to pH. Recent data are compatible with pH-taxis being a component of social motility. The physiological relevance, in the context of trypanosome metabolism and pH gradients within the tsetse host, will be discussed. These results also raise the possibility that tissue tropism within mammals is due, at least in part, to chemotaxis.

1Shaw & DeMarco et al. (2019). doi: 10.1038/s41467-019-08696-y

4C A bar-seq fitness screen of Leishmania CRISPR-Cas9 knockout mutants shows the importance of motility in colonization of sandflies

Beneke, Tom Smith, James (University of Oxford, Sir William Dunn School of Pathology); Hookway, Edward (Research Department of Pathology, University College London); Becvar, Tomas; Myskova, Jitka; Lestinova, Tereza; Sadlova, Jovana; Volf, Petr (Department of Parasitology, Faculty of Science, Charles University); Wheeler, Richard (Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford); Gluenz, Eva (University of Oxford, Sir William Dunn School of Pathology)

The number of fully sequenced Leishmania genomes increases steadily but the function of more than half of the predicted genes remains unstudied. To accelerate dissection of gene function in Leishmania spp. we developed a streamlined pipeline for generation of CRISPR-Cas9 mediated gene knockout in which each mutant carries a unique 17-nucleotide barcode, allowing loss-of-function screens and phenotyping of mutants in mixed populations. To date we have used this method to generate more than 400 null mutants in a knockout screen of the flagellar proteome. This mutant library enables a systematic analysis of the contribution of conserved and kinetoplastid-specific proteins to flagellar motility and function. Individual phenotyping uncovered diverse phenotypes including defects in motility, flagellar assembly, axonemal structure, paraflagellar rod (PFR) integrity and maintenance of cell shape. We show here how pooled fitness screens were used in vitro and in infections of the permissive sand fly vector Lutzomyia longipalpis to determine the effect of flagellar defects on parasite growth and ability to colonise the fly. Promastigotes that were aflagellate, paralysed or uncoordinated swimmers were severely diminished in the parasite population after defecation of the bloodmeal. Dissection of sand flies infected with L. mexicana lines lacking the central pair protein PF16 showed that these paralysed promastigotes did not reach the anterior regions of the fly alimentary tract. These results suggest that motility in Leishmania parasites is vital for colonization of sand flies and show the potential of this method for larger-scale fitness screens of Leishmania mutants.

4D Dramatic changes in gene expression in different forms of Crithidia fasciculata reveal potential mechanisms for insect-specific adhesion in kinetoplastid parasites

Povelones, Megan L. Filosa, John (University of Pennsylvania); Berry, Corbett (Drexel University); Ruthel, Gordon (University of Pennsylvania); Dudkin, Elizabeth (Penn State Brandywine); Povelones, Michael (University of Pennsylvania)

Pathogenic kinetoplastid species have diverse life cycles. One common feature is the ability to adhere to tissue in their insect host. The adherent structure resembles a hemidesmosome and may be important for parasite replication and development within the insect. Monoxenous parasites of insects, such as Crithidia fasciculata, also adhere to insect tissue. In addition, C. fasciculata can also form attachments on non-living substrates, allowing the process of adhesion to be studied in culture. We are investigating C. fasciculata as a model for insect-specific adhesion in kinetoplastids. We have conducted transcriptomic analysis of swimming nectomonads and adherent haptomonads generated in culture and compared them to gene expression profiles of parasites in the hindgut of Aedes aegypti mosquitoes. We have discovered large changes in transcript abundance between swimming and adherent cells even in culture, indicating that the adherent form is indeed a distinct developmental stage. We have also identified possible signal transduction pathways that may act to maintain these cell states. Furthermore, by comparing the genes upregulated in cultured adherent and mosquito hindgut-adherent parasites, we have identified a large number of candidate adhesion genes. These include members of the gp63 family, which are developmentally regulated in Leishmania and have been implicated in adhesion in other kinetoplastids. We are working on CRISPR/Cas9-mediated gene editing to facilitate functional analysis of candidates. We have also used dual-RNAseq to address the mosquito's response to infection with C. fasciculata. Intriguingly, we found a small number of genes that are differentially regulated in infected hindguts, some of which have functions in mosquito innate immunity.

4E The generation of advanced human skin tissue models allows the controlled investigation of developmental processes of tsetse-transmitted African trypanosomes in the dermis

Reuter, Christian Finger, Tamara; Fey, Philipp; Krüger, Timothy; Walles, Heike; Groeber-Becker, Florian; Engstler, Markus

African trypanosomes are transmitted by the tsetse fly through deposition of the infective metayclic stage parasites into the mammalian skin. Our knowledge about the early stages of human trypanosome infection at the site of the insect's bite remains limited. Therefore, we propose bioartificial human skin as versatile model system for the investigation of tsetse-borne trypanosome infections. We have developed novel primary human skin equivalents with improved mechanical properties. Our skin model resembles native human skin in its histological architecture and distinctive physiology. We have used tsetse flies to successfully simulate the natural infection process by direct transmission of trypanosomes to our articifal skin models. High-end 4D imaging of the injection site revealed an unexpected complextity of the skin lesions, with direct implications on the early dissemination of the trypanosomes within the host dermis. Most importantly, the parasites complete their natural developmental progamme within the human skin model. We found a very rapid transition from the transmitted quiescent metacyclic trypanosomes to proliferative slender bloodstream forms, and further differentiation to fly-transmissible stumpy forms. We have also documented the interaction of trypanosomes with dermal fibroblasts, and have embarked on an extensive dual RNA-Seq analysis of trypanosome infections of primary human skin models.

4F A Trypanosoma brucei factor H receptor and its mechanism of interaction with the mammalian complement system

Macleod, Olivia JS Bart, Jean-Mathieu (UMR INTERTRYP-CIRAD); MacGregor, Paula (University of Cambridge); Peacock, Lori (University of Bristol); Hester, Svenja (University of Oxford); Rust, Steven (MedImmune); Ravel, Sophie (UMR INTERTRYP-CIRAD); Trevor, Camilla (University of Cambridge); Winter, Ralph (MedImmune); Mohammed, Shabaz (University of Oxford); Gibson, Wendy (University of Bristol); Vaughan, Tristan J (MedImmune); Taylor, Martin C (London School of Hygiene and Tropical Medicine); Higgins, Matthew K (University of Oxford); Carrington, Mark (University of Cambridge)

The macromolecular interactions betweenTrypanosoma brucei and its mammalian host are mediated through trypanosome proteins present on the external face of the plasma membrane. The interactions characterized to date are the clearance of immunoglobulin bound to variant surface glycoprotein and receptor mediated endocytosis of host transferrin, haptoglobin haemoglobin and the primate-specific innate immunity factor TLF1. The complexity of the interaction is likely to be far greater. A GPI-anchored surface receptor has been identified that binds mammalian host complement factor H (FH). FH is a large glycoprotein of 155 kDa that regulates the innate immune system by inhibiting the alternative complement pathway and preventing cell lysis. A number of diverse microorganisms have evolved mechanisms of recruiting FH to avoid destruction by this pathway. Here, we have characterized the trypanosome FH receptor bound to ligand at a molecular level by determining the structure using x-ray crystallography and identifying a pocket at the membrane distal end of the receptor that binds domain 5 of FH, leaving domains 1-4 free to inactivate complement C3. The nature of this receptor-ligand interaction represents a novel form of binding to FH compared to other microorganisms. In cultured trypanosomes, the receptor is expressed in bloodstream and procyclic forms. The receptor has been localized to the cell surface. In infected tsetse flies, FH receptor expression is maintained on the cell surface of trypomastigotes in the midgut and proventriculus, where T. brucei would be exposed to complement in the blood meal. The role of the FH in infection was investigated using a FH receptor knockout cell line and tracking parasitemia in a mouse model and transmission to the tsetse fly. Taken together, these observations are the first evidence of a direct interaction between the complement system and T. brucei, represent a further mechanism of immune evasion in mammalian blood, and demonstrate a key advancement our understanding of trypanosome receptor functioning.

4G Baboon apolipoprotein L-1 forms pH-independent membrane channels, thus conferring immunity to the human pathogen T. b. gambiense

Verdi, Joey Thomson, Russell; Raper, Jayne (Hunter College CUNY)

Humans and baboons are immune to T. b. brucei infection through the antimicrobial activity of apolipoprotein L-1 (APOL1), a primate specific protein that forms cation-selective channels in lipid membranes after endocytosis. The human pathogen T. b. gambiense resists lysis by human APOL1 by preventing channel formation in a mechanism that is thought to involve an increase in endolysosomal membrane stiffness, relative to T. b. brucei, thus preventing the physical insertion of APOL1 into the membrane. However, the Papio hamadryas baboon ortholog of APOL1 can kill T. b. gambiense parasites, prompting the hypothesis that it can insert into lipid membranes more readily than human APOL1. To investigate this, we used an in vitro planar lipid bilayer system to determine the environmental membrane insertion requirements for both the human and baboon APOL1 proteins. While the human APOL1 can only insert into lipid membranes once acidified, the P. hamadryas ortholog inserts into membranes at both acidic and neutral pH, suggesting that it could insert into T. b. gambiense membranes prior to reaching the 'stiffened' endolysosomal membranes. We then tested whether the baboon APOL1 orthologs from the other baboon species shared this same property and determined that, similar to the human variant, the T. b. gambiense susceptible P. cynocephalus baboon APOL1 variant requires pH acidification to efficiently form cation channels. The P. cynocephalus variant was correspondingly less lytic to trypanosome parasites in vitro than the unrestricted P. hamadryas protein, suggesting that membrane insertion properties are an important mediator of T. b. gambiense immunity in baboons.

4H Structure of the trypanosome transferrin receptor reveals mechanisms of ligand recognition and immune evasion

Trevor, Camilla Gonzalez-Munoz, Andrea (MedImmune); Macleod, Olivia (University of Cambridge); Woodcock, Peter (University of Oxford); Rust, Steven; Vaughan, Tristan (MedImmune); Garman, Elspeth (University of Oxford); Minter, Ralph (MedImmune); Carrington, Mark (University of Cambridge); Higgins, Matthew (University of Oxford)

The transferrin receptor (TfR) is the mediator of iron acquisition in bloodstream form T. brucei. The heterodimeric TfR is encoded by the ESAG6 and ESAG7 genes, present in each of the ~14 bloodstream expression sites. There has been diversification in these genes across expression sites, which has been hypothesised to favour host species specificity to accommodate different transferrins from the wide mammalian host range. We expressed two divergent transferrin receptors and measured the affinities of different mammalian transferrins for these receptors. We find no evidence of emerging specificity, with one receptor exhibiting broad promiscuity whilst the other shows reduced binding to transferrin from all species tested. We used structural studies to locate variable residues and resolved a 2.65A structure of the receptor in complex with human transferrin. TfR adopts a fold strikingly similar to characterised trypanosome membrane proteins based on a three-helix bundle. Mapping of the receptor-ligand interface shows limited sequence variation within the receptor binding pocket. Rather, we find that polymorphic residues reside predominantly in exposed surfaces that are not shielded by N-linked glycans. We propose that evolutionary divergence in the transferrin receptor repertoire has arisen through an advantage conferred in counteracting the host adaptive immune system, possibly a slow motion antigenic variation favouring long term persistence.

SESSION V:
Cell Cycle/Cytokenisis
Michele Klingbeil, Chair.
Tuesday 8:45 am
04/30/19

5A Protein kinases regulating kinetochore function in Trypanosoma brucei

Mottram, Jeremy; Saldivia, Manuel (University of York); Rao, Srinivasa (Novartis Institute for Tropical Diseases)

The kinetochore is a macromolecular structure that assembles on the centromeres of chromosomes and provides the major attachment point for spindle microtubules during mitosis. In Trypanosoma brucei the proteins that make up the kinetochore are highly divergent, with the inner kinetochore comprising at least 20 distinct and essential proteins (KKT1-20) that include four protein kinases, CLK1 (KKT10), CLK2 (KKT19), KKT2 and KKT3. We performed a phenotypic screen of T. brucei bloodstream forms with a Novartis kinase-focused inhibitor library, which identified a number of selective inhibitors with potent pan-kinetoplastid activity. Deconvolution of an amidobenzimidazole series using a selection of 37 T. brucei mutants that over-express known essential protein kinases identified CLK1 as the primary target. Biochemical studies show that the irreversible competitive inhibition of CLK1 is dependent on a Michael acceptor forming an irreversible bond with C215 in the ATP binding pocket, a residue that is not present in human CLK1, thereby providing an explanation for its specificity. Chemical inhibition of CLK1 impairs inner kinetochore recruitment and dramatically compromises cell cycle progression, leading to cell death. We show that KKT2 is a substrate for CLK1 and identify phosphorylation of S508 to be essential for KKT2 function and for kinetochore assembly. We propose that CLK1 is part of a novel signalling cascade that controls kinetochore function via phosphorylation of the inner kinetochore protein kinase KKT2. This work highlights a novel drug target for trypanosomatid parasitic protozoa and a new chemical tool for investigating the function of their divergent kinetochores.

5B Analysis of the interface between microtubules and unconventional kinetochores

Hayashi, Hanako Llauró, Aida; Bailey, Megan E. (Department of Physiology and Biophysics, University of Washington); Wilson, Alex; Ludzia, Patryk (Department of Biochemistry, University of Oxford); Höög, Johanna (Department of Chemistry and Molecular Biology, University of Göthenburg); Asbury, Charles L. (Department of Physiology and Biophysics, University of Washington); Akiyoshi, Bungo (Department of Biochemistry, University of Oxford)

During chromosome segregation, kinetochores interact with dynamic spindle microtubules to facilitate the movement of chromosomes toward spindle poles. Although kinetochore components are widely conserved among diverse eukaryotes, kinetoplastids possess a unique set of kinetochore proteins, which have no significant homology to conventional kinetochore proteins. It remains unclear how the unconventional kinetoplastid kinetochore attaches to spindle microtubules. To address this question, we analysed the localisation of KKT (kinetoplastid kinetochore) proteins in Trypanosoma brucei and found that KKT4 localizes not only at kinetochores but also on spindle microtubules during metaphase. In vitro single molecule imaging demonstrated that KKT4 can directly bind microtubules and track dynamic microtubule tips. By testing truncated KKT4 proteins in a microtubule cosedimentation assay, we identified its microtubule-binding domain, which lacks similarity to other microtubule-binding proteins. Knockdown of KKT4 in vivo caused lagging kinetochores during anaphase and the deletion mutant of KKT4 that lacks the microtubule-binding domain failed to rescue the knockdown phenotype, showing that the microtubule-binding domain of KKT4 is important for proper chromosome segregation. Taken together, we propose that KKT4 forms a primary element of the kinetochore–microtubule interface in Trypanosoma brucei. To understand the structural basis of the kinetoplastid kinetochore-microtubule interface, we are currently using high-pressure freezing/freeze substitution and electron tomography on mitotic trypanosome cells. Our preliminary 3D ultrastructural reconstruction has visualized kinetochore-like structures positioned close to microtubules in the mitotic nucleus.

5C Organising the cell cycle in the absence transcriptional control: Dynamic phosphorylation co-ordinates the Trypanosoma brucei cell cycle post-transcriptionally

Urbaniak, Michael D. Benz, Corinna (Lancaster University)

The temporal control of protein involved in the regulation and progression of the Trypanosoma brucei cell cycle is essential not only to co-ordinate correct growth and cell division, but also for successful transmission between hosts. Regulation of the eukaryotic cell cycle is typically achieved largely through transcriptional regulation, but despite a paucity of transcription factor mediated gene expression, T. brucei regulates it transcript and protein abundance over the cell cycle.

Here we present an unbiased approach to investigate the role of dynamic phosphorylation in the post-transcriptional regulation of T. brucei cell cycle. Combining centrifugal elutriation to produce high quality cell cycle synchronised populations (1) with SILAC based quantitative proteomics (2) allowed the identification of 917 cell cycle regulated phosphorylation sites (≥ 3-fold change) and 443 cell cycle regulated proteins (≥ 1.5-fold change). The changes in phosphorylation status observed were of a larger magnitude, more numerous, and largely independent from the changes at the protein level. The cell cycle regulated phosphoproteins identified included those known roles in the cell cycle (i.e. CDKs, KKTs, PUF9), along with many other novel candidates.

Clustering of co-regulated phosphorylation sites according to their cell cycle profile reveals a trend of increasing phosphorylation over the cell cycle, with significant enrichment ($P < 0.01$) of GO terms relating to the cell cycle and mRNA binding. Endogenous in situ tagging was used to verify cell cycle regulation of selected phosphoproteins, and their essentiality examined using inducible RNAi, focusing on RNA binding proteins including the CSBPII homologues RBP33 and RBP45. This data provides an insight into the role of dynamic phosphorylation in co-ordinating the post-transcriptional regulation of the cell cycle.

1. Benz C, Dondelinger F, McKean PG, Urbaniak MD. Cell cycle synchronisation of Trypanosoma brucei by centrifugal counter-flow elutriation reveals the timing of nuclear and kinetoplast DNA replication. Sci Rep. 2017;7(1):17599.

2. Urbaniak MD, Martin DM, Ferguson MA. Global quantitative SILAC phosphoproteomics reveals differential phosphorylation is widespread between the procyclic and bloodstream form lifecycle stages of Trypanosoma brucei. Journal of proteome research. 2013;12(5):2233-44.

5D Nucleopores complexes are essential in the maintenance of ploidy in Trypanosomatid parasites.

Sterkers, Yvon Yagoubat, Akila; Stanojcic, Slavica (University Montpellier); Berry, Laurence; Crobu, Lucien (CNRS); Kuk, Nada; Morelle, Christelle; Lachaud, Laurence; Bastien, Patrick (University Montpellier)

Trypanosoma brucei and Leishmania spp. exhibit many original molecular and cellular features, among which a closed mitosis which remains poorly known. In terms of ploidy, while T. brucei is diploid, Leishmania spp. display mosaic aneuploidy aneuploidy demonstrated by FISH and confirmed by NGS. The biological significance of mosaic aneuploidy is still poorly understood but it has been associated with drug resistance and environment adaptability. Nuclear pore complexes (NPCs) are large multiprotein channels embedded in the nuclear envelope and made of nucleoporins. Although NPCs' primary function is to regulate bidirectional nucleo-cytoplasmic exchanges of macromolecules, recent research indicates that nucleoporins are involved in other core processes. Using tagged proteins, we determined that Mlp1 was located at the nuclear basket of the NPCs, while Mlp2 was intranuclear in interphasic cells and relocated at the poles of the mitotic spindle during mitosis. In T. brucei, RNAi knockdown of TbMlp2 had no effect on cellular growth whereas Mlp1 appears to be an essential gene. However, the use of flow cytometry and FISH revealed that both RNAi knockdowns perturbed the distribution of chromosomes during mitosis, leading to aneuploidy. The phenotypes observed here are in agreement with the hypothesis that Mlp2 plays a role in chromosomal segregation and might be involved in the constitution of kinetochores while Mlp1 would play a role in the regulation of replication. Indeed, in TbMlp1 knockdown, the vast majority of cells divided asymmetrically and in all dividing nuclei, the total number of homologs was an odd number (i.e: 1:2 or 2:3).The role of Mlp1 in DNA replication will be further explored by DNA molecular combing and Short Nascent Strand Sequencing (SNS-seq).

5E Cell cycle checkpoint proteins HUS1 and RAD9 differentially modulate gene amplification and genome variability in Leishmania

Obonaga, Ricardo Damasceno, Jeziel Dener (University of Glasgow); L. Reis-Cunha, João (Universidade Federal de Minas Gerais(UFMG)); V. Santos, Elaine (University of São Paulo(FMRP-USP)); L. A. Silva, Gabriel; Virgilio, Stela (University of São Paulo (FMRP-USP)); S. Bastos, Matheus (University of São Paulo); C. Bartholomeu, Daniella (Universidade Federal de Minas Gerais (UFMG)); McCulloch, Richard (University of Glasgow); Tosi, Luiz Ricardo Orsini (University of São Paulo (FMRP-USP))

Our knowledge of the mechanisms leading and controlling the remarkable genome plasticity in Leishmania, manifested as gene and chromosome copy number variation (CNV and CCNV), is still limited. Here we used cell lines deficient in the 9-1-1 complex subunits HUS1 and RAD9, involved in the early steps DNA replication stress detection and signalling, to examine the involvement of these cell cycle checkpoint factors in these phenomena. While HUS1 deficient cells were defective in promoting CNV of linear amplicons of the DHFR-TS locus, deficiency of RAD9 led to exacerbated CNV of circular amplicons of this locus. This pattern was seen in both non-stressed and replication-stressed conditions. Also, whole genome sequencing showed increased CCNV and SNP accumulation in HUS1-deficient cells, while the opposite was observed in the RAD9-deficient cell line. These differences correlated with distinct patterns of DNA damage resolution, as measured by γH2A levels, and DNA synthesis during cell cycle progression of HUS1 and RAD9 deficient cell lines. Altogether, our data indicate that (i) the cell cycle checkpoint pathway has a role in modulating genome plasticity in Leishmania; (ii) despite being part of the same complex, HUS1 and RAD9 have distinct effects on the structure of amplified elements during CNV; and (iii) the role of HUS1 and RAD9 in genome plasticity is not only restricted to CNV events, but is pervasive throughout the genome, including the modulation of CCNV events.

5F Robust loss-of-fitness measurements, combinatorial RNAi and genome-wide epistatic interactions by Direct RNAi Fragment-sequencing (DRiF-Seq)

Wickstead, Bill Brusini, Lorenzo; D'Archivio, Simon; Stretton, Owen; Gadelha, Catarina (University of Nottingham)

RNA interference target-sequencing (RIT-seq) is one of the most important developments in trypanosome research of the last decade. It has a proven track-record in applications where strong positive selection can be used to isolate rare RNAi mutants conferring growth advantage under specific conditions. However, as a measure of loss-of-fitness (LoF), RIT-Seq data perform poorly against “ground truth” estimates from individual knockdowns or pools of RNAi mutants, and up to 20% of genes confer an unlikely gain-of-fitness on knockdown. These factors prevent RIT-Seq screens for conditional LoF (rather than growth advantage) or applications looking for modifiers of fitness, such as gene epistasis.

Here we present a new method for robust LoF measurement at genome-scale. DRiF-Seq differs from RIT-Seq in fragment library, recipient cells, site of integration, and also method of sequencing and data analysis. The combined method monitors growth of 100,000s of RNAi mutants as individual clones while accounting for sources of variation through “model agnostic” a posteriori noise estimation. Two independent genome-wide libraries of >200,000 clones demonstrate strong correlation in LoF, excellent recapitulation of experimental data, and an estimated positive predictive value ~20-fold that of RIT-Seq. Additionally, following individual fragments reveals which regions of genes produce LoF.

We have used DRiF-Seq to quantitatively map fitness in components of cell division and combined with a KKIP7 mutant in the first genome-wide screen for epistasis in trypanosomes (eDRiF-Seq), demonstrating both negative and positive epistatic interactions at the kinetochore. The technology can be applied to combinatorial RNAi libraries to study gene redundancy (doubleDRiF-Seq) and is portable between strains – as shown by application to a 250,000 clone library in pleomorphic EATRO1125 cells.

5G Long-term Trypanosoma brucei live-cell imaging using agarose micro-chambers identifies novel RNAi phenotypes and protein localization during cell division

de Graffenried, Christopher L. Muniz, Richard; Sladewski, Thomas (Brown University); Renner, Lars (Leibniz Institute of Polymer Research); Perry, Jenna (Brown University)

Long-term live cell imaging of Trypanosoma brucei would provide unique insights into how the parasite divides and allow the establishment of direct causal relationships between RNAi phenotypes as they emerge over time. However, T. brucei must remain motile to divide, which has made it difficult to observe individual cells for entire cell cycles at high spatial and temporal resolution. To address this issue, we have developed a live-cell imaging platform using agarose micro-chambers cast from a PDMS stamp that allows viable procyclic cells to be confined in small volumes and imaged continuously for up to 36 hours. We have used the chambers with a microscope that employs focal plane detection and simultaneous DIC/fluorescence imaging to observe cells dividing twice at magnifications ranging from 40X to 100X. To test this system, we have triggered RNAi against the important cytokinetic regulators TbPLK and TOEFAZ1 and identified novel phenotypic stages that were not evident in our previous fixed-cell analyses. Using fluorescently tagged marker proteins, we have also monitored duplication of cytoskeletal structures such as the hook complex and proteins such as the kinesin KLIF, which migrates inward along the cleavage furrow at late stages of cell division. Future refinements of our live-cell imaging approach will allow the simultaneous imaging of wild-type and RNAi-depleted cells within the same chamber and the monitoring of cell division in other T. brucei life cycle stages.

TT5H Characterising Leishmania kinetochores with XL-BioID

Geoghegan, Vincent Jones, Nathaniel; Dowle, Adam; Larson, Tony; Mottram, Jeremy (University of York)

Kinetochores are large complexes that connect chromosomes to microtubules ensuring faithful chromosome segregation during mitosis. In kinetoplastids, many of the proteins that form the inner kinetochore have no apparent homology to kinetochore proteins from other eukaryotes. During a kinome-wide screen for protein kinases important in Leishmania differentiation and intracellular survival, the kinetochore proteins KKT2, KKT3 and KKT10 appeared to be essential. We therefore focussed on these kinases to understand their role in the Leishmania cell cycle. As a large chromatin attached protein complex, the kinetochore is challenging to characterise biochemically and has not been directly analysed in Leishmania. We have developed an enhanced proximity biotinylation based method, XL-BioID, for greater coverage of large protein complexes by mass spectrometry. We applied XL-BioID to investigate interacting partners of KKT2, KKT3 and KKT10. Our method provided substantially increased coverage of Leishmania kinetochores compared to a standard proximity biotinylation workflow or co-immunoprecipitation, identifying 98 high confidence proximal proteins from the inner through to the outer kinetochore. From our mass spectrometry data, we identified new components of the kinetoplastid kinetochore and carried out co-localisation microscopy confirming their role at the kinetochore or mitotic spindle. We were also able to calculate relative intra-complex distances between proteins, enabling us to build a topological model of the Leishmania kinetochore. Our data provide detailed insight into the unique kinetoplastid kinetochore and XL-BioID will be applicable to the study of other protein complexes in a variety of organisms.

TT5I Identifying novel factors associated with trypanosome DNA replication forks using nascent DNA proteomics

Rocha-Granados, Maria Bermudez, Yahaira; Dodard, Garvin (University of Massachusetts); Gunzl, Arthur (UConn Health); Klingbeil, Michele M. (University of Massachusetts, Amherst, Amherst, MA)

DNA replication, transcription and chromatin remodeling are coordinated to ensure accurate duplication of genetic and epigenetic information. Trypanosomatid parasites such as Trypanosoma brucei display unusual DNA replication properties including significantly fewer origins of replication than model eukaryotes, a highly divergent Origin Replication Complex (ORC), and an apparent lack of several replication factor homologs. Recent studies in T. brucei indicate functional links among DNA replication, transcription, and antigenic variation, but the underlying mechanisms remain unknown. Here, we adapted an unbiased technology for the identification of replication fork proteins called iPOND (isolation of proteins on nascent DNA) to T. brucei, its first application to a parasite system. This led to the mass spectrometric identification of core replication machinery and of proteins associated with transcription, chromatin organization, and DNA repair that were enriched in the vicinity of an active replication fork. Among the enriched proteins, DNA polymerase a and replication factor C were scoring in the top, around 25% of the proteins identified were of unknown function and, therefore, have the potential to be essential trypanosome-specific replication proteins. Initial characterization of a protein annotated as a Replication Factor C subunit (Tb927.10.7990), and a protein of unknown function (Tb927.3.5370) revealed that both proteins retain nuclear localization throughout the cell cycle. While Tb927.3.5370 appeared to be a dispensable gene, Tb927.10.7990 proved to be essential since its silencing caused a growth defect in procyclic cells, accumulation of zoids and impaired DNA replication. Future studies on the generated proteins list can contribute to the understanding of DNA replication dynamics in T. brucei and how replication is coordinated with other cellular processes to maintain genome integrity.

SESSION VI:
Gene Expression
Ruslan Afasizhev, Chair.
Tuesday 2:00 pm
04/30/19

6A A Novel PNUTS-PP1 Phosphatase Complex Orchestrates RNA Pol II Transcription Termination in T. brucei

Kieft, Rudo Zhang, Yang (Department of Biochemistry and Molecular Biology, University of Georgia); Marand, Alexandre P. (Department of Genetics, University of Georgia); Moran, Jose Dagoberto (Department of Biochemistry and Molecular Biology, University of Georgia); Bridger, Robert; Wells, Lance (Complex Carbohydrate Research Center, University of Georgia); Schmitz, Robert J. (Department of Genetics, University of Georgia); Sabatini, Robert (Department of Biochemistry and Molecular Biology, University of Georgia)

O-linked glucosylation of thymine in DNA (base J) represents an epigenetic mark, regulating Pol II transcription termination within polycistronic gene clusters in Leishmania and T. brucei. Loss of base J leads to altered transcription termination and read-through transcription. The mechanism of termination in kinetoplastids, including the role of base J, is unknown.However, in humans and yeast, the transition from transcription elongation to termination involves dephosphorylation of the carboxy-terminal domain of Pol II by PP1 protein phosphatase as part of a multimeric complex composed of regulatory subunits PNUTS, Tox4, Wdr82 and PP1. In L. tarantolae we have identified a similar complex, including PP1, potential homologues of PNUTS and Wdr82, and a novel J-DNA binding protein (JBP3), presumably a functional homologue of the Tox4 DNA binding protein. In T. brucei we have verified the individual interactions of the multimeric complex, as well as the association of PNUTS and JBP3 with chromatin. Similar to the effects from the loss of J and H3.V, ablation of PNUTS, Wdr82 and JBP3 by RNAi leads to read-through transcription at Pol II termination sites and de-repression of Pol I transcribed VSG expression sites. We also detect increased transcription at regions between divergent Pol II promoters, presumably resulting from bi-directional promoter activity where non-productive RNA synthesis (antisense) is regulated by early termination. Similar increased anti-sense transcription at promoters is seen upon depletion of Wdr82 and PNUTS in human cells. Our data suggests a model where base J (via JBP3) recruits the PP1 complex to transcription termination sites at promoters and 3'end of gene clusters. Mechanism of Pol II transcription and impact upon DNA replication will be discussed.

6B Genome-wide mapping reveals R-loop activity in genome maintenance at sites of pre-mRNA processing in Leishmania.

Damasceno, Jeziel Dener Reis-Cunha, João L (Universidade Federal de Minas Gerais(UFMG)); Briggs, Emma; Lapsley, Craig; Crouch, Kathryn (University of Glasgow); Bartholomeu, Daniella C. (Universidade Federal de Minas Gerais(UFMG)); McCulloch, Richard (University of Glasgow)

RNA-DNAhybrids (R-loops) can be resolved by RNase H enzymes in vivo and have been implicated in transcription and replication processes,as well as in telomere and chromatin functioning. If left unresolved, R-loops can be a source of genome instability. Here, we used DNA:RNA ImmunoPrecipitation followed by deep sequencing (DRIP-seq) to characterize the genome-wide distribution of R-loops in a cell line engineered to perform DiCre-mediated inducible knockout (KO) of RNase H1. Like in T. brucei, R-loops were enriched at promoters of rRNA and splice leader genes and within the regions between coding sequences, coinciding with polyadenylation and/or splicing sites and areas of low nucleosome occupancy. However, unlike in T. brucei, R-loop abundance was proportional to chromosome size and showed strongest enrichment towards the centre of each chromosome. In addition, we find that at least a subset of R-loops co-localizes with nascent DNA synthesised at intergenic and UTR regions. Whole genome sequencing after RNase H1 KO induction revealed a significantly increased rate of mutations at the sites of R-loop accumulation, and evidence for increased levels of chromosome copy number variation. Thus, our data indicate that: 1) the most widespread sites of R-loop localization in Leishmania correlate with pre-mRNA processing and are mutation-prone when R-loops persist; 2) global R-loop distribution reflects variation in genome architecture across kinetoplastid parasites; and 3) impaired R-loop resolution is associated with promotion of genome variability. Currently, we are analysing DRIP-seq data to determine the genome-wide distribution of R-loops in WT and RNAseH1 depleted cells.

6C EpiRNomics in trypanosomatids: impact on parasite cycling between the mammalian and insect host

Michaeli, Shulamit Rajan, K. Shanmugha (Bar-Ilan University); Chikne, Vaibhav (Bar-Ilan Unversity); Cohen Chalamish, Smadar (Bar-Ilan university); Doniger, Tirza (Bar-Ilan University); Sirvastava, Ankita (University of Connecticut Health Center); Unger, Ron (Bar-Ilan University); Gunzl, Arthur (University of Connecticut Health Center); Tschudi, Christian (Yale University)

Genome wide mapping of pseudouridine (Ψ)s in T. brucei using Psi-seq revealed that rRNA is hypermodified in bloodstream form (BSF) trypanosomes (Chikne et al., Sci. Rep. 2016). Mutations introduced by CRISPR-Cas9 in the pseudouridine synthase CBF5 of small nucleolar (sno)RNAs that guides these modifications abolished transformation of procyclic to bloodstream forms (BSF) upon RBP10 overexpression, suggesting that proper pseudouridylation is a pre-requisite for developmental cycling of trypanosmes. We will present whole genome-wide mapping of Ψ on small RNAs by RNA-seq and mass-spectrometry. Ψs were not only found on snRNAs, but also on many other non-coding RNAs, such 7SL RNA, vtRNA, C/D and H/ACA snoRNAs. Our analysis revealed the highest number of Ψ on snRNAs reported thus far in nature. We provide evidence that Ψs strengthen RNA-RNA interactions and control RNA-protein interactions, especially at elevated temperatures. All the single hairpin loop sn/snoRNAs can potentially guide modification on both rRNA and snRNAs. This unique dual functionality enables simultaneous regulation of both the spliceosome and ribosome while cycling between the two hosts. Additionally, we will present high throughput mapping of 2'-O-methylation on rRNA that revealed 97 Nms in procyclics, similar to the number found in humans. Of these twenty sites are hypermodified in BSF. Silencing of snoRNAs that guide abundant modifications on rRNA and are located near the exit tunnel of the ribosome, halted rRNA processing at the last step, suggesting a regulatory link between RNA modification and rRNA processing. Accordingly, in trypanosomes, unlike in humans or yeast, depletion of individual critical modifications on rRNA affected ribosome biogenesis and growth of the parasite.

6D The extended family of retrotransposon hotspot genes in trypanosomes: junk DNA or doing things differently?

Florini, Francesca Naguleswaran, Arunasalam (IZB, University of Bern, Switzerland); Gharib, Walid H. (Interfaculty Bioinformatics Unit, University of Bern, Switzerland); Bringaud, Frédéric (Laboratoire de Microbiologie Fondamentale et Pathogénicité (MFP), Université de Bordeaux, France); Roditi, Isabel (IZB, University of Bern, Switzerland)

Retrotransposon hotspot (RHS) proteins constitute 7 sub-families of (predominantly) nuclear proteins. They are found exclusively in the trypanosome clade, and they are encoded by a large multigene family that consists of 118 genes or pseudogenes in T. brucei TREU927. Because of their complexity and abundance, RHS have often been discarded from transcriptomic and proteomic analyses and this, together with the absence of homology to any known protein, made it challenging to predict their functions. Nevertheless, members of the RHS family were found to be associated with RNA polymerase II (Pol II) and with the chromatin remodeller TbRRM1.

We recently showed that RHS2, RHS4 and RHS6 sub-families are required for transcription. ChIP analyses demonstrated that these 3 RHS cover Pol II transcription units, that their binding is reduced in Pol I transcription units (e.g. the procyclin loci) and absent from silent regions of the genome. In addition, these RHS bind spliced leader (SL) genes, which are also transcribed by Pol II. Depletion of RHS reduces SL expression and results in the accumulation of unspliced, but polyadenylated RNAs in the nucleus. Establishment of global run-on sequencing (GRO-Seq) for the first time in trypanosomes demonstrated a global requirement for RHS in RNA synthesis by Pol II. These results demonstrate that trypanosomes have evolved specific factors to control their unconventional transcription.

Further experiments indicate that the RHS5 sub-family is not essential in procyclic forms and that RHS1 knockdown gives a different phenotype than RHS2, 4 and 6. Moreover, the RHS7 sub-family is expressed only in the salivary glands of tsetse, raising the possibility that RHS are differentially required during the life cycle.

6E Iron-dependent control of transferrin receptor expression in Trypanosoma brucei

Tienqwe, Calvin J Cornell, Lucy; Gilabert-Carbajo, Carla; Lai, Zhihao (Imperial College London); D. Bangs, James (University at Buffalo)

Iron is an essential co-factor for many enzymatic reactions but can be very toxic at high concentrations. Consequently, mammalian cells exert a tight control of intracellular iron. Iron regulatory RNA-binding protein (IRPs) bind iron responsive elements (IRE) to modulate stability of mRNAs encoding major components of the iron-acquisition pathway, such as the transferrin receptor (mTfR).

In T. brucei, an evolutionarily divergent transferrin receptor (TbTfR) mediates uptake of host transferrin as a nutritional source of iron. Like mTfR, TbTfR mRNA stability is modulated by iron availability. However, previous work indicates its regulation does not follow the IRP/IRE paradigm (Fast et. al. 1999), although it is mediated via TbTfR 3'-UTR (Benz et al, 2018). The T. brucei trans-acting IRP(s) await discovery.

To search for T. brucei factors regulated by iron-availability, BSF trypanosomes were starved for transferrin (and consequently iron) by TbTfR RNAi. Among the upregulated proteins identified following RNA-sequencing was a previously uncharacterised RNA-binding protein (TbIRP-1). By quantitative-PCR and immunoblotting, we show that TbTfR and TbIRP-1 mRNA and protein levels are co-ordinately upregulated following transferrin starvation and treatment with the iron chelator deferoxamine. Subcellular fractionation and immunofluorescence show that TbIRP-1 is cytosolic.

Overexpression of TbIRP-1 results in upregulation of TbTfR mRNA and protein, suggesting TbIRP-1 may stabilise TbTfR mRNA under iron-limiting conditions. TbIRP1 overexpression leads to accumulation of cells with aberrant nuclei and kinetoplasts, cell cycle arrest and consequently rapid cell death within 24 hrs suggesting that TbIRP-1 levels may be tightly regulated. Furthermore, we show that TbIRP-1 is not autoregulatory, propose that it regulates a subset of genes with an iron-related function, and studies are underway to characterise its mechanism.

6F Trypanosome brucei RNase H2 is an essential nuclear enzyme with roles in both transcription initiation and antigenic variation.

Briggs, Emma Crouch, Kathryn; Lemgruber, Leandro (WCIP, University of Glasgow); Hamilton, Graham (Glasgow Polyomics); Lapsley, Craig; McCulloch, Richard (WCIP, University of Glasgow)

Incorporation of ribonucleotides into the DNA genome threatens genome integrity and frequently results in DNA damage. Ribonuclease (RNase) H enzymes of two classes are found in most organisms and act to remove such ribonucleotides, either by excising ribonucleotides that are embedded into the DNA strand, or hydrolysis of RNA that has become base-paired to one strand of the DNA helix creating R-loop structures. Both RNase H1 and RNase H2 are essential for mammalian development, although yeast is able to tolerate the loss of both enzymes. Trypanosoma bruceiRNase H1-null mutants are viable in culture, and only localised DNA damage is found in the variant surface glycoprotein (VSG) transcription units, which correlates with increased levels of VSG expression changes. Using RNAi, we demonstrate that loss of the RNase H2 catalytic subunit (TbRH2A) is lethal, resulting in cell cycle arrest and massive accumulation of DNA damage. Genome-wide mapping revealed the highly localised formation of lesions at RNA Polymerase (Pol) II transcription initiation sites, with an associated loss of R-loops, after TbRH2A depletion. Furthermore, differential gene expression analysis revealed changes in both RNA Pol I and Pol II transcribed genes, including those which may relate to changes in cytosolic nucleic acid that have been documented in mammalian RNase H2 mutants. Lastly, we observed increased R-loop and DNA damage levels in the VSG transcription units, along with increased expression of normally inactive VSGs. Thus, we have documented divergent roles for the two nuclear RNase H enzymes in the RNA Pol II transcribed core genome of T. brucei, yet functional overlap during RNA Pol I-mediated antigenic variation.

6G Histone variants link genome architecture and antigenic variation in Trypanosoma brucei

Cosentino, Raul O Müller, Laura SM; Brink, Benedikt; Siegel, T. Nicolai (Department of Veterinary Sciences, Experimental Parasitology. Ludwig-Maximilians-Universität München)

T. brucei avoids the elimination by the immune system of its mammal host by antigenic variation.

This mechanism involves the expression of only one Variant Surface Glycoprotein (VSG) gene isoform at any given time and the periodical switching among a large repertoire of diverse VSG isoforms, frequently, by recombination.

Chromatin conformation affects both, gene expression and recombination, but it is unknown if it plays a role in the antigenic variation mechanism of T. brucei.

To address this, we performed genome-wide chromosome conformation capture (Hi-C) experiments, which revealed a striking partitioning of the T. brucei genome with subtelomeric regions, encoding silent VSG arrays, folding into distinct, highly compacted compartments.

Furthermore, using a combination of Hi-C, FISH, ATAC-seq and single cell RNA-seq analyses, we observed that the deletion of histone variants H3.V and H4.V increases DNA accessibility within VSG expression sites and the clustering among them, which lead to switching of the expressed VSG via homologous recombination. Thus, identifying histone variants as a molecular link between global genome architecture, local chromatin conformation and antigenic variation.

Finally, we also explored the presence of other specific trans-interactions among genomic loci. The observed interaction pattern and the possible implications will be discussed.

TT6H An ORF-based whole-genome gain-of-function library for Trypanosoma brucei

Quinn, McKenzie Gomez, Stephanie (The George Washington University); Schultz, Danae (Harvey Mudd College); Kim, Hee-Sook (Cleveland State University); Hovel-Miner, Galdriel (The George Washington University)

Despite decades of active discovery in all areas of trypanosome research, more than 50% of the Trypanosoma brucei genome is annotated as hypothetical genes of unknown function. Further progress in understanding both the pathogenesis and basic biology of Trypanosome species requires the development of versatile approaches for genome-scale functional analysis. A whole-genome RNAi loss-of-function library has proved instrumental in the identification of new genetic functions and pathways in T. brucei. Previously, use of shotgun cloning and digested genomic DNA have been implemented in the formation of whole-genome overexpression libraries with some success. To better capture the majority of T. brucei gene full open reading frames (ORFs), we have produced an ORF-based whole-genome gain-of-function library that contains >90% of the targeted genes. The library is induced in the context of a genetic screen to isolate cells resulting in a desired phenotype. Following a genetic screen, next-generation sequencing libraries are enriched using a unique oligo that targets the gain-of-function library for PCR amplification and Illumina platform sequencing. Preliminary experiments returned insufficient read depth and suggested that further optimization of sequencing libraries was required. To this end, we developed a qPCR method for assessing the amount of gain-of-function specific DNA in each next-generation library preparation. Assessment of library quality by qPCR enabled further optimization of sequencing libraries, through modifications to the enrichment PCR, and more accurate pooling of samples for Illumina sequencing. This tool was then applied to a preliminary screen for the identification of genes associated with melarsoprol resistance, in which a specific melarsoprol resistant population was isolated following induction of the gain-of-function library. The tools and optimizations made for gain-of-function library implementation will be discussed as well as early findings from a melarsoprol genetic screen.

TT6I UTR-seq: hundreds of new regulatory 3'-untranslated regions in African trypanosomes

Trenaman, Anna Wall, Richard; Horn, David (University of Dundee)

Trypanosomatids constitutively transcribe almost all genes polycistronically and utilise RNA-binding proteins (RBPs) to modulate gene expression post-transcriptionally. RBPs often act by binding mRNA 3' untranslated regions (UTRs) thereby underpinning major environmental and developmental 'regulons'. Relatively few regulatory UTRs have been identified, however, and specific sequences and RBPs that bind them remain largely uncharacterised. We recently identified a respiratome regulon under the control of the Trypanosoma brucei ZC3H39/40 RBP and characterised this regulon using transcriptomics, interactomics and proteomics. Seeking further regulons, we developed UTR-seq, a genomic-scale screening platform to profile regulatory UTRs. We assembled and validated a dual positive/negative reporter and then cloned a deep-coverage library of 1-3 kbp genomic DNA fragments immediately downstream of the reporter. A bloodstream stage T. brucei library was then assembled and screened; using high-throughput sequencing of cloned UTR fragments. All known regulatory UTRs reported the expected behaviour in the published literature (aldolase, GPI-PLC, cytochrome oxidase, glucose transporters, procyclin, for example). In addition, we identify hundreds of further regulatory UTRs. Specific enrichment of 'sense-oriented' UTR-fragments indicated that post-transcriptional regulatory capacity typically resides within these regions and is strand-specific. The dataset reveals regulatory capacity within specific segments of annotated UTRs, and significant cohorts of positive regulatory UTRs associated with genes encoding RBPs, VSGs, glycolytic/gluconeogenic enzymes, translation initiation factors, chaperones and cell cycle regulators. Negative regulatory UTRs are associated with genes encoding cohorts of mitochondrial ribosome subunits, enzymes involved in lipid metabolism and kinases. Thus, UTR-seq reveals the relevant regulatory segments that comprise probable regulons, supporting the long-held view that many such regulons operate, and also suggests candidate cognate RBPs.

TT6K Characterization of putative chromo- and SET-domain transcription regulators in Trypanosoma brucei

Staneva, Desislava Carloni, Roberta; Auchynnikava, Tatsiana; Tong, Pin; Matthews, Keith; Allshire, Robin (The University of Edinburgh)

In eukaryotic nuclei, DNA is packaged into chromatin by histone proteins. Specific post-translational modifications (PTMs) on the histones result in the formation of distinct chromatin domains which regulate gene expression by permitting or repressing transcription. In many eukaryotes, transcription is the most important step of gene regulation as this is when the cell commits resources to produce a protein or an RNA. This general model might not apply to Trypanosoma brucei which is thought to regulate its gene expression predominantly post-transcriptionally. Nevertheless, numerous putative chromatin modifiers can be found in the T. brucei genome but their role in activating or repressing transcription remains largely unknown.

We have systematically identified putative readers, writers and erasers of trypanosome histone PTMs, YFP-tagged 68 of these proteins, determined their localization and performed ChIP-seq analysis to identify chromatin interactors. Surprisingly, many of these proteins were enriched at transcription start regions, suggesting a potential role in transcriptional regulation. Although most candidates were present broadly throughout promoter regions, two proteins in particular, the chromo-domain protein Chromo1 and the SET-domain protein Set27, displayed sharper overlapping peak profiles. Moreover, co-IP and mass spectrometry revealed that Chromo1 and Set27 strongly interact with each other. We propose that Set27 writes a histone methyl mark that is read by Chromo1 and are currently testing this model by in vitro histone binding and methyltransferase assays as well as mass spectrometry to identify histone PTMs enriched in Chromo1 IPs. More broadly, we aim to determine how the candidate writers, readers and erasers influence transcriptional regulation in T. brucei and how this may change during the developmental cycle of this parasite.

TT6L Intriguing roles assigned to Leishmania eIF4E paralogs by structure-function studies and CRISPR-Cas 9-mediated knockout approach

Shapira, Michal Tupperwar, Nitin; Shrivastava, Rohit; Kamus-Elimeleh, Dikla; Orr, Irit (Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel); Meleppattu, Shimi; Leger-Abraham, Melissa (Microbiology Department, Blavatnik Institute, Harvard Medical School, Boston, MA); Wagner, Gerhard (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA)

Translation regulation plays a key role in the differentiation processes of trypanosomatids. As such, great efforts have been invested in dissecting the roles of the various cap-binding complexes in these organisms, given how such complexes drive and/or repress global or gene-specific translation in higher eukaryotes. The genomes of leishmanias and trypanosomes encode six different orthologs of the cap-binding translation factor eIF4E and five eIF4Gs with the latter serving as a "hub" protein for the pre-initiation complex (PIC) formation during translation initiation. A network of IF4E-interacting proteins comprising Leish4E-IP1 and Leish4E-IP2 regulate the differential activities of cap-binding proteins during the organism's life cycle. Leish4E-IP1 is a repressor of LeishIF4E-1's function. The 3-D structure of LeishIF4E-1, in complex with a fragment of Leish4E-IP1, was recently determined by X-ray crystallography. A null LeishIF4E-1(-/-) mutant depleted of both LeishIF4E-1 alleles was generated using CRISPR-Cas9 technology. While the mutant cells showed impaired translation and a severe reduction in growth rates, the most apparent cellular effect was monitored at the point of switching between the amastigote and promastigote stage. This result coincides with the transcriptomic analysis data of LeishIF4E-1 associated mRNAs, which are enriched with transcripts involved in the biogenesis of the cytoskeleton and the flagellum. A similar CRISPR-Cas9-based approach was employed for LeishIF4E-3, another eIF4E paralog that during nutritional stress concentrates in granules that mostly store stalled ribosomal particles and mRNAs until conditions improve. The knock out attempts resulted in a heterologous LeishIF4E-3 (-/+) mutant, in which only a single allele was eliminated. The level of LeishIF4E-3 expression in this mutant was reduced, affecting its morphological plasticity and responsiveness to changing conditions. As such, the CRISPR-Cas9-based knock-out strategy highlighted unexpected and intriguing functions of the different LeishIF4E proteins.

SESSION VII:
Pathogenesis II
Megan Povelones, Chair.
Wednesday 8:45 am
05/1/19

7A Leishmania RNA Viruses and Parasite Virulence

Beverley, Stephen M. Akopyants, Natalia; Lye, Lon-Fye (Washington University School of Medicine)

Leishmania in South America often bear the dsRNA Leishmaniavirus (LRV1). Like most Totiviruses, LRV1 is neither shed nor infectious, and thus may be viewed as a persistent endobiont. Perspectives on the importance of protozoal viruses changed upon discovery that L. guyanensis LRV1 is associated with hypervirulence and increased metastasis in animal models, the latter being a hallmark of the more severe forms of leishmaniasis (Ives et al. Science 2011). We have been pursuing this observation intensively as a new paradigm of protozoal virulence. For Leishmania we developed RNA interference tools for reproducibly generating isogenic lines lacking LRV1s (Brettmann et al PNAS 2016). This has allowed extension of findings with L.guyanensis to L. braziliensis, the predominant agent of mucocutaneous leishmaniasis (MCL). An important question is the contribution of LRV1 with Leishmania pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models, which is complicated by several factors. Recently we showed that the presence of LRV1 was associated with increased relapse and/or treatment failures in human L.braziliensis-infected patients treated with pentavalent antimonials in Peru and Bolivia, as well as in L.guyanensis infections treated with pentamidine (Adauai et al & Bourreau et al. J. Inf. Dis 2016). The association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of tegumentary disease caused by Leishmania sp.

We have embarked on a systematic survey of known and new viruses in Leishmania as well as their monoxenous insect trypanosomatid relatives, using a wide range of methods including next-gen RNA sequencing (Grybchuk et al PNAS 2018). This has greatly expanded our knowledge of the trypanosomatid virome with the discovery of multiple new viruses. The properties, evolution and potential contributions of these to virulence and biology will be discussed.

7B Unconventional activation of a DYRK kinase implicated in the Trypanosoma brucei quorum-sensing response

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

A genome-wide RNAi screen for molecules required for stumpy formation in trypanosomes identified a proposed YAK kinase family member (Tb927.10.15020; Mony et al, Nature 2014). However, closer phylogenetic inspection revealed that this gene encodes a divergent member of the DYRK kinase family, molecules implicated in cellular quiescence and stress responses in diverse eukaryotes. To gain insight into the function and regulation of TbDYRK, T. brucei null and ectopic expression mutants have been constructed and recombinant protein produced in insect cells. Using a combination of pCPTcAMP-based phenotypic screening and in vitro kinase assays we demonstrate the essential role in kinase activity of the molecule's unconventional DFS motif, as well as the unusual histidine in the HxY motif of the activation loop.

In parallel, the phosphoproteome of a TbDYRK null mutant has been compared to parental cells, identifying molecules reproducibly regulated in the absence of the kinase. A kinase assay was also performed on parasite lysates confirming the direct interaction of some of the effectors with TbDYRK and their involvement in differentiation to stumpy forms. For example, the negative regulator of transcription NOT5 was identified as a substrate of TbDYRK potentially inactivated by phosphorylation. Indeed, NOT5 knock-down leads parasites to differentiate prematurely in mice. Conversely, TbDYRK phosphorylates the zinc finger protein ZC3H20, an mRNA regulator that promotes for stumpy formation. These results suggest that TbDYRK can both inhibit 'slender retainer' proteins and activate of 'stumpy promoter' molecules by phosphorylation, driving differentiation. This provides new insight into the signaling pathways regulating stumpy formation, revealing novel regulatory mechanisms and molecular components that control parasite development.

7C Metabolic sensing in African trypanosomes: A possible role of aquaglyceroporins and glycerol along the entire parasite life cycle

CALVO ALVAREZ, ESTEFANIA Pineda, Erika (Centre de Résonance Magnétique des Systèmes Biologiques (CRMSB), Université de Bordeaux); Cren-Travaillé, Christelle (Trypanosome Transmission Group, Trypanosome Cell Biology Unit, Institut Pasteur); Biran, Marc (Centre de Résonance Magnétique des Systèmes Biologiques (CRMSB), Université de Bordeaux); Crouzols, Aline (Trypanosome Transmission Group, Trypanosome Cell Biology Unit, Institut Pasteur); Bastin, Philippe (Trypanosome Cell Biology Unit, Institut Pasteur); Bringaud, Frédéric (Laboratoire de Microbiologie Fondamentale et Pathogénicité, Centre de Résonance Magnétique des Systèmes Biologiques, Université de Bordeaux); Rotureau, Brice (Trypanosome Transmission Group, Trypanosome Cell Biology Unit, Institut Pasteur)

African trypanosomes are extracellular protists responsible for sleeping sickness. These parasites are exclusively transmitted by the bite of the tsetse fly. In the mammalian host, *Trypanosoma brucei* accumulates in the interstitial spaces of several tissues, including brain, visceral adipose tissue and skin[1-3]. The bloodstream forms of *Trypanosoma brucei* rely on glucose as a main carbon source. However, the skin and adipose tissues are rich in glycerol that can also be used by trypanosomes to feed gluconeogenesis even in the presence of glucose[4,5]. Aquaglyceroporins (AQPs) are protein channels responsible for the transport of water and small uncharged solutes such as glycerol. African trypanosomes express three distinct AQP differentially localized at their surface. To assess whether glycerol could support the proliferation and survival of trypanosomes in the tsetse digestive tract and in the extravascular niches of the mammalian host, we generated pleomorphic null mutants lacking all aquaglyceroporins but expressing a bioluminescent and fluorescent reporter in order to monitor their development in the insect as well in a mouse model. *In vitro,* mutant parasites were viable but failed to utilize glycerol. *In vivo*, trypanosomes deprived of AQPs were able to fully complete their cyclical development within the insect vector, differentiating into transmissible metacyclic forms in the salivary glands. However, in the mammalian host, although their parasitemia remained similar to that of their parental strain, AQP1-3 null mutants were impaired to disseminate in the extravascular compartment of the skin. We propose that the absence of aquaglyceroporins might prevent these mutants to sense a possible glycerol attractant signal and / or to adapt their metabolism to the glycerol-rich micro-environment of the mammalian host skin."

7D Glycerol: a tissue-specific stumpy inducing factor in trypanosomes?

Guegan, Fabien Teixeira, Alexandra; Machado, Henrique (Instituto de Medicina Molecular); Calvo Alvarez, Estefania; Rotureau, Brice (Pasteur Institut); Figueiredo, Luisa (Instituto de Medicina Molecular)

Transmission of Trypanosoma brucei depends on a quorum sensing mechanism, which triggers an irreversible differentiation into quiescent stumpy forms. Our laboratory recently showed that the adipose tissue is a major parasite reservoir in mice. Whether and how parasites differentiate into stumpy forms in non-vascular spaces remains unknown. A T. brucei infection promotes the activation of lipolysis in adipocytes, in which triglycerides are catabolized and secreted in the form of fatty acids and glycerol. We hypothesized that glycerol could play an important role in trypanosome differentiation. First, we showed that when exposed to exogenous glycerol in culture, parasites differentiate prematurely into stumpy forms. Stumpy forms induced by glycerol exposure are GFP::PAD1 positive, cell cycle arrested in G1/G0 and transmissible to the insect vector. When we measured the glycerol concentration in the medium of T. brucei in vitro culture, we found that the concentration of secreted glycerol increases with parasite density. Interestingly, parasites impaired for glycerol production (by depletion of glycerol kinase genes), also prematurely differentiate to stumpy forms, pointing to glycerol metabolism as an important hub for parasite differentiation. Mice infected with this mutant parasite line shows very low parasitemia and all mice resolved the infection. We propose that depletion of glycerol kinase and exposure to glycerol may result in the accumulation of glycerol-3-phosphate inside the parasites, triggering differentiation into stumpy forms. Given that glycerol is small, soluble, heat-stable and triggers differentiation to stumpy forms, we propose it may act as a stumpy inducing factor, especially in tissues where its concentration is higher, such as an adipose tissue actively consuming its lipid stores.

7E A coordinated expression of TbMYND and RBP6 allows development of Trypanosoma brucei infectious forms in the tsetse

Casas-Sanchez, Aitor Lopez-Escobar, Lara; Cansado-Utrilla, Cintia; Haines, Lee (Liverpool School of Tropical Medicine); Darby, Alister (University of Liverpool); Shamsani, Jannah; Walrad, Pegine (University of York); Acosta-Serrano, Alvaro (Liverpool School of Tropical Medicine)

The colonization and migration of Trypanosoma brucei through the tsetse is accompanied by a series of developmental changes that are the result of a tightly-regulated programmed gene expression. After the 'stumpy' bloodstream forms differentiate into the procyclic stage in the midgut lumen, the parasites colonize the midgut ectoperitrophic space via the proventriculus (PV). Later in the PV, they further differentiate into the short epimastigote form, which then establishes a salivary gland infection that leads to the formation of transmissible metacyclic trypomastigotes.

To identify T. brucei genes involved in life cycle progression in the tsetse, we used RNA-seq to compare the expression profiles of proventricular trypanosomes from a fly-transmissible strain with that of a strain unable to infect salivary glands. Among the top up regulated hits, we identified RNA-binding protein 6 (RBP6) known to trigger differentiation of procyclics into metacyclics when overexpressed in vitro, and a hypothetical protein conserved across kinetoplastids, with a predicted MYND zinc finger domain. While all kinetoplastid homologues conserve the MYND domain, only the primitive Bodo saltans homolog has an additional RNA-binding domain, suggesting that TbMYND may act as an effector accessory protein. Interestingly, overexpression of TbMYND in procyclic cells led to the formation of epimastigotes in vitro via a secreted factor, and restored the infectivity of salivary glands in the impaired strain. While TbMYND is primarily expressed in PV forms, RBP6 is expressed in both PV and salivary gland forms. Thus, we hypothesize that the sequential expression of TbMYND and RBP6 is essential to coordinate development in the tsetse. We are currently identifying putative binding partners of TbMYND and RBP6 and assessing the fly infection phenotype of TbMYND KO cells.

7F Two cold shock domain proteins in Trypanosoma brucei are involved in the developmental regulation of metacyclic VSG genes

Toh, Justin Nkouawa, Agathe; Rojas-Sánchez, Saúl; Shi, Huafang; Lei, Yuling; Kolev, Nikolay G. (Yale School of Public Health); Toh, Justin (Yale School of Public Health, New Haven, CT); Tschudi, Christian (Yale School of Public Health)

The development of Trypanosoma brucei to forms infectious to mammals consists of several transitions between life-cycle stages, accompanied with changes in gene expression. Two of these developmental steps are characterized with the presence of distinct surface coat proteins, epimastigote cells in the tsetse fly's salivary glands express brucei alanine-rich protein (BARP) and infectious metacyclics are coated with a variant surface glycoprotein (VSG). To further our understanding of trypanosome development, we used our RBP6-driven in vitro metacyclogenesis system to perform a targeted RNAi screen for a selection of 82 genes shown previously to change in expression between procyclics and metacyclics and/or to affect bloodstream form cells development to stumpy forms. We analyzed the effects of RNAi against the gene targets on BARP and metacyclic VSG expression, as well as kinetoplast repositioning to epimastigote configuration. Our screen revealed 24 genes that were required for at least one of these three steps in the generation of metacyclics. To complement the results of the RNAi screen, we inducibly expressed all the nucleic acid binding proteins from the screen individually in procyclic T. brucei cells and again assessed kinetoplast repositioning, BARP, and metacyclic VSG expression. The zinc finger protein ZC3H45 and two proteins with a predicted cold shock DNA/RNA-binding domain, which we named CSD1 and CSD2, triggered a rapid activation of metacyclic VSGs expression. Interestingly, expression of CSD1 resulted in high levels of VSG expression within 16 hours of induction, and this effect was achieved even in the absence of RBP10, a gene which is required for RBP6-triggered metacyclogenesis. Our data demonstrate that CSD1 is a critical regulator of metacyclic VSG expression during T. brucei development and suggest that this protein may play a direct role in the activation of VSG coat synthesis and infectivity acquisition.

7G BCAAs reshape the metabolism and infection capability of Trypanosoma cruzi metacyclic trypomatigotes

de Freitas Nascimento, Janaína da Silva Damasceno, Flávia; Nakamura Rapado, Ludmila; Barison, Maria Julia; Marsiccobetre, Sabrina (Universidade de Sao Paulo); de Luna Vitorino, Francisca Nathália (Instituto Butantan); Merino, Emilio Fernando (Virgina Tech); Cassera, Maria Belen (Virginia Tech); Pinheiro Chagas da Cunha, Julia (Instituto Butantan); Silber, Ariel Mariano (Universidade de Sao Paulo)

Differentiation to the infective non-replicative form metacyclic trypomastigote happens at the final portion of insect vector's gut and it is crucial to the progress of Trypanosoma cruzi life cycle. Factors that play a role in metacyclogenesis include temperature, pH and nutritional stress followed by the availability of specific metabolites. Our results show that some amino acids such as Pro, His and Gln are able to fully support cell differentiation whereas the BCAAs completely block it. In addition, a combination of the "pro-metacyclogenic" amino acid Pro with one of the "anti-metacyclogenic" BCAAs produces viable metacyclics, however, with a drastically reduced infectivity. Aiming to investigate the peculiarities of metacyclics differentiated in the presence of BCAAs, we combined proteomics and metabolomics, comparing metacyclics obtained in triatomine artificial urine (TAU) supplemented with Pro combined or not with Leu, Ile, and Val. Results show that when compared to the metacyclics originated in TAU-Pro, 40 proteins are differentially regulated in cells differentiated in TAU-Pro-Leu, whereas 131 and 179 are differentially regulated in TAU-Pro-Ile and TAU-Pro-Val, respectively. Among those, 22, 11 and 13% of the proteins are putatively related to metabolic processes, respectively. Generally, both glycolysis and TCA cycle are reduced in metacyclics obtained in the presence of Pro-BCAAs whereas amino acid and purine metabolic pathways are increased. Specifically, metacyclics obtained in the presence of Pro-Ile and Pro-Val have increased lipid metabolism as well as redox metabolism. Furthermore, evidence supports that gluconeogenesis is active in metacyclics obtained in TAU-Pro-Val. Together, the data point that the impact of BCAAs on metacyclogenesis in reshaping cell metabolism can contribute to the reduced infectivity observed in these parasites.

POSTERS: Session A
Sunday 7:00 pm
04/28/19

1 - TT1G TURNOVER OF VSG IN TRYPANOSOMA BRUCEI
Garrison, Paige Umaer, Khan; Bush, Peter; Bangs, Jay (University at Buffalo)

2 - TT1H How DNA breaks contribute to mosaic VSG formation in Trypanosoma brucei?
Romero-Meza, Gabriela Mugnier, Monica

3 - TT2H Targeting the ubiquitin system of Leishmania Mexicana
Burge, Rebecca Damianou, Andreas; Catta-Preta, Carolina (The University of York); Rodenko, Boris (UbiQ); Mottram, Jeremy C. (The University of York)

4 - TT2I Identifying highly divergent glycosyltransferases in the African Trypanosome
Duncan, Samuel Damerow, Manuela; Ferguson, Michael (The University of Dundee)

5 – TT3F Swim like your lifecycle depends on it: Investigating chemotaxis and navigation in Leishmania parasites
Walrad, Pegine B. Findlay, Rachel; Osman, Mohamed (York Biomedical Research Institute); Gadelha, Hermes (Department of Mathematics); Kaye, Paul (York Biomedical Research Institute); Rogers, Matthew (London School of Hygeine and Tropical Medicine); Wilson, Laurence (Biological Physical Sciences Institute, University of York)

6 – TT3G The dual FAZ domain-localizing protein FAZ27 cooperates with FLAM3 and ClpGM6 to control morphology transitions in Trypanosoma brucei
AN, TAI ZHOU, QING; HU, HUIQING; LI, ZIYIN

7 - TT3H TrypTag: Biological lessons and opportunities from a molecular atlas of trypanosomes
Dean, Samuel Billingdon, Karen (University of Oxford); Halliday, Clare (Oxford Brookes); Madden, Ross (University of Oxford); Carrington, Mark (University of Cambridge); Hertz-Fowler, Christiane (University of Liverpool); Vaughan, Sue (Oxford Brookes); Gull, Keith (University of Oxford); Sunter, Jack (Oxford Brookes); Wheeler, Richard (University of Oxford)

8 - TT3I High frame rate multi-colour and multi-focal plane microscopy to gain new insights into trypanosomatid parasite swimming
Wheeler, Richard J.

9 - TT3K UNRAVELLING THE ROLE OF PROTEIN KINASES IN LEISHMANIA DIFFERENTIATION
Baker, Nicola Catta-Preta, Carolina; Neish, Rachel; Powell, Ben; Ferreira, Eliza; Geoghegan, Vincent; Newling, Katherine; Pitchford, John; Mottram, Jeremy (University of York)

10 A ‘HUB AND SPOKE’ model for the nuclear lamina in trypanosomes

Padilla-Mejia, Norma E; Koreny, Ludek; Zoltner, Martin; Field, Mark (University of Dundee)

The nuclear lamina supports multiple roles in the nucleus, including structural integrity, chromatin organisation and developmental gene expression, for which correct assembly is essential. Recently it emerged that there are multiple lamina systems across eukaryotes, and despite evidence for independent descent, these systems have remarkably analogous functions, indicating a fundamental conserved role. In Trypanosoma brucei several elements of the unique lamina have been identified. Two, NUP-1 and NUP-2, comprise essential lamina components and both are large coiled-coil proteins, indicating distinct architecture from the metazoan lamin system. We exploited a set of deletion mutants of NUP-1 to reveal organisational principles of the trypanosome lamina in vivo. We find that both the N- and C-termini of NUP-1 act as interaction hubs, and that disruption of hub assembly impacts additional components of the lamina and the nuclear envelope. We also identified an interaction with the nuclear pore complex protein, NUP98, and a new lamina component, with a prenylation motif highly reminiscent of the metazoan lamin structure. Both terminal domains of NUP-1 play a role in assembly of the trypanosome lamina and suggest a ‘hub and spoke’ architecture.

11 A fluorescent reporter strain designed to screen for factors that initiate surface remodeling in the African trypanosome

Schulz, Danae; Walsh, Matty; Naudzius, Ellie (Harvey Mudd College)

Our group is interested in molecular mechanisms that drive adaptation to distinct host environments in Trypanosoma brucei. In order to adapt to a host change, the parasite undergoes dramatic changes in morphology, metabolism, and surface protein composition. We previously showed that chromatin interacting bromodomain proteins are important for maintaining the bloodstream form of the parasite. Small molecule inhibition of bromodomain proteins causes changes in the transcriptome that mirror many of those that occur during the transition from the bloodstream form to the insect stage procyclic form. The physiological effect of small molecule bromodomain inhibition is loss of bloodstream-specific Variant Surface Glycoprotein (VSG), gain of insect-stage specific procyclin protein on the cell surface, and decreased virulence in a mouse model of infection. We hypothesize that the loss of VSG and gain of an invariant epitope on the cell surface might cripple the parasite's bloodstream-specific immune evasion mechanisms, thus leading to a decrease in virulence. To explore whether other small molecule inhibitors could be used to induce transcription of insect-stage procyclin in bloodstream cells, we generated a reporter strain to easily detect transcription of procyclin by flow cytometry. We replaced one allele of the procyclin-encoding EP1 gene with GFP, flanking it with native EP1 5' and 3' UTR sequences. We first verified that GFP expression was low in bloodstream cells and high in procyclic cells. We also showed that GFP expression increases in bloodstream cells upon induction of differentiation and following treatment with small molecule bromodomain inhibitors. We then screened a library of 1,531 FDA approved small molecules to ask whether any induced GFP expression in bloodstream cells. We found that eflornithine, phenothiazine, and spironolactone all had this effect. This screen provides proof of principle that our reporter can be used to screen other epigenetic-targeted small molecule libraries. We also hope to use our reporter for genetic screens aimed at identifying molecular factors that are important for initiating transcription of EP1 downstream of the signaling pathways that transduce environmental signals from the cell surface.

12 A heterodimer of a kinetoplastid-specific kinesin and its catalytically inactive partner associates with the microtubule quartet of Trypanosoma brucei

Hashimi, Hassan; Müller, Nora; Kaltenbrunner, Sabine (Faculty of Science, University of South Bohemia, Ceské Budejovice, Czechia); Varga, Vladimír (Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czechia); Lukeš, Julius (Institute of Parasitology, Biology Center, Czech Academy of Sciences, Ceské Budejovice, Czechia)

The cytoskeleton, a fundamental component of the eukaryotic cell, is comprised of highly conserved components. Among them are the tubulin-based microtubules, which form a network within the cytosol, and kinesin motor proteins that move along the microtubule surface, carrying cargo to the plus ends of these polymers. Despite conservation of their core parts, cytoskeletons throughout eukaryotes adopt different forms and functions as shaped by evolution. For example, the subpellicular microtubule corset and microtubule quartet, a structure that runs from the basal body along the flagellar attachment zone (FAZ), are unique features of kinetoplastids. Also, the kinesin that is a topic of this study, TbKIFx, and its presumably catalytically inactive partner TbPH1, are also restricted to kinetoplastids. TbKIFx/TbPH1 interaction is strong, persisting under conditions of high ionic strength needed to disassemble the microtubule corset that confines these two proteins. Because the latter property indicates they associate with microtubules, isolated cytoskeletons were probed for the presence of epitope-tagged TbKIFx. Indeed, TbKIFx gives a signal that is enriched with both the basal body and FAZ, suggesting they may interact with the microtubule quartet. Immunogold labeling of tagged TbKIFx visualized by scanning electron microscopy further supports this conclusion. Interestingly, we observe that phosphorylated tyrosines are also enriched in the FAZ. Because TbPH1 is named for the pleckstrin homology domain, which has been reported to bind phosphorylated tyrosines, we hypothesize that a TbKIFx/TbPH1 may interact with proteins bearing this modification, perhaps carrying them along the microtubule quartet.

13 A new type of extended-synaptotagmin (E-Syt) in Trypanosomes

Stepinac, Emma; Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna); He, Cynthia (Department of Biological Science, National University of Singapore); Bonhivers, Melanie; Robinson, Derrick R. (CNRS, Microbiologie Fondamentale et Pathogénicité); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna)

Extended-synaptotagmins (E-Syts) are resident proteins of the endoplasmic reticulum (ER), anchoring on the ER membrane via their N-terminal transmembrane domain. They interact with the plasma membrane via their C-terminal tandem C2 domains (three to five), and transport glycerolipids between the two membranes via a unique SMP domain in their central region. E-Syts are widely present in various organisms and have a more general function than the synaptotagmins (Syts), which serve to facilitate calcium-trigged exocytosis by tethering secretory vesicles to the plasma membrane.

We have recently identified an E-Syt-like protein in Trypanosoma brucei (TbE-Syt), which is conserved throughout Trypanosome species. TbE-Syt is predicted to contain an N-terminal transmembrane domain and a characteristic SMP domain. However, unlike all other known E-Syts, TbE-Syt contains only two C2 domains. Our in vivo immunofluorescence studies confirm that TbE-Syt localizes to the ER through its N-terminal membrane anchor. Bioinformatics analyses suggest that the putative SMP domain forms a homodimeric cylindrical β-barrel with a highly hydrophobic channel capable of lipid binding as seen in the E-Syt2 crystal structure. We further determined a 1.5-Å resolution crystal structure of its second C2 domain, which reveals two stably bound Ca2+ ions coordinated by four conserved D, E, or N residues within two spatially adjacent loops. Such loops mediate Ca2+-dependent membrane binding of TbE-Syt, as demonstrated by our liposome binding assays.

Additionally, we found that TbE-Syt also binds membrane in another Ca2+-independent manner via a positively charged surface patch. Overall, our data suggest that TbE-Syt is a new E-Syt with a unique domain arrangement distinct from all known E-Syts, which might confer its specific regulation and/or function in Trypanosomes.

14 A single dose of antibody-drug conjugate cures a stage 1 model of African trypanosomiasis.

MacGregor, Paula; Gonzalez-Munoz, Andrea; Jobe, Fatoumatta (MedImmune); Taylor, Martin (London School of Hygiene and Tropical Medicine); Rust, Steven; Sandercock, Alan (MedImmune); Macleod, Olivia (University of Cambridge); Van Bocxlaer, Katrien; Francisco, Amanda (London School of Hygiene and Tropical Medicine); D'Hooge, Francois; Tiberghien, Arnaud; Barry, Conor; Howard, Philip (Spirogen); Higgins, Matthew (University of Oxford); Vaughan, Tristan; Minter, Ralph (MedImmune); Carrington, Mark (University of Cambridge)

The trypanosome haptoglobin-haemoglobin receptor (HpHbR) has been exploited as a route of uptake for an antibody-drug conjugate (ADC) that is completely effective against Trypanosoma brucei in a standard stage 1 mouse model of infection. Recombinant human anti-HpHbR monoclonal antibodies were isolated and shown to be internalised in a receptor-dependent manner. Antibodies were conjugated to a pyrrolobenzodiazepine (PBD) toxin and killed T. brucei in vitro at picomolar concentrations. A single therapeutic dose (0.25 mg/kg) of a HpHbR antibody-PBD conjugate completely cured a T. brucei mouse infection within 2 days with no re-emergence of infection over a subsequent time course of 77 days.

These experiments provide a demonstration of how developments in oncology ADCs can be exploited to treat protozoal diseases that desperately require new therapeutics.

15 Absence of hydrodynamic flow mediated sorting of surface bound complexes in non-African trypanosomes

Hambleton, Isobel Kelly, Steven (University of Oxford); Carrington, Mark (University of Cambridge)

There are hundreds of species of Trypanosoma species which survive in the bloodstream of vertebrate hosts. A small number of African trypanosome species have evolved an ornate system of antigenic variation to avoid recognition and clearance from the host bloodstream. In addition, several African trypanosomes are known to passively sort antigen-immunoglobulin complexes, resulting from hydrodynamic flow, to the posterior end of the cell, effectively concentrating them in the flagellar pocket where they are endocytosed. Here, we have asked the question whether this self-cleaning process was present in the ancestor of African trypanosomes or has it evolved subsequent to the VSG coat? We have measured the ability of two trypanosome species, Trypanosoma theileri and Trypanosoma carassii to remove surface bound biotin-streptavidin and biotin-IgG complexes. T. theileri is an extracellular Stercorarian parasite of cattle and is in a sister group to African trypanosomes, whereas T. carassii infects carp and diverged before the split between the Stercorarian and Salivarian trypanosomes. Here, we show that neither the accumulation of surface bound IgG towards the posterior pole nor rapid endocytosis occurs in either species. This suggests that the IgG does not protrude sufficiently to be subjected to hydrodynamic flow forces and/or the GPI anchored cell surface proteins do not have the same ability to diffuse over the cell surface as VSG. The observations also suggest that the VSG coat has allowed the African trypanosomes to evolve a self-cleaning surface in addition to its role in antigenic variation.

16 Assessing the structure and function of trypanosome lytic factors in human breast milk

Ebenezer, Karen; Raper, Jayne (CUNY Hunter College, Department of Biological Sciences); Thomson, Russel; Verdi, Joseph (CUNY Hunter College)

Breast milk is the primary source of nutrition for newborns, containing a range of cholesterol, nutrients, and innate immune factors that protect infants from infection. Human milk hosts a range of immune factors, such as active complement proteins and maternally transferred IgA-2 antibodies, but also has significant lipid character. High-density lipoproteins (HDL) complexes circulate in plasma, and transport cholesterol and lipid throughout the human body. These complexes are characterized by the presence of the structural cholesterol acceptor protein apolipoprotein A-1 (APOA1). Recently human breast milk proteome analyses have indicated the presence of APOA1; however, the relative amount of HDL in breast milk remains unclear and its presence has not been validated by conventional biochemistry. Additionally, our innate immune system is fortified by a specific subset of high-density lipoproteins, called trypanosome lytic factors (TLFs), named for their ability to kill an infective unicellular parasite called the African trypanosome. These TLFs contain two unique proteins in addition to APOAI: Haptoglobin-related protein (HPR) and apolipoprotein L-1 (APOL1), the latter of which induces the death of a trypanosome through pore formation. Till now, APOL1 has not been detected in any human breast milk proteome analyses, despite the known presence of APOA1. Using various purification procedures and biochemical assays, we investigated whether TLF complexes were present in human breast milk, whether it possessed a detectable degree of functional APOL1, and whether the presence of these innate immune factors can edify our current understanding of infant immune systems.

17 Blastocrithidia, a trypanosomatid with all three stop codons reassigned

Lukes, Julius); Nenarokova, Anna; Zahonova, Kristina; Horakova, Eva; Kachale, Ambar; Nenarokov, Serafim (Biology Centre); Yurchenko, Vyacheslav (University of Ostrava); Paris, Zdenek (Biology Centre)

Blastocrithidia spp., a clade of insect trypanosomatids, reassigned all three stop codons to encode amino acids. Only one of them (UGA) has a dual meaning, since it acts as a sense codon in some cases and as a termination codon in others. It is thus hard to reconcile this ambiguity with how protein synthesis termination, one of the most basic cellular processes, operates in this parasitic flagellate. While the mechanisms behind this extreme reassignment remain unclear, Blastocrithidia, which is availabe in culture, may represents a suitable model system for studying codon reassignments, as a wide range of methods of forward and reverse genetics, available for the related Trypanosoma and Leishmania spp., is also likely to function in Blastocrithidia. Moreover, unlike ciliates, which are well-known for stop codon reassignment, all known kinetoplastids aside from the genus Blastocrithidia have the canonical nuclear genetic code.

We have sequenced and analyzed the genomes, transcriptomes and proteomes of Blastocrithidia sp. and Leptomonas jaculum, its closest relative with the canonical genetic code. We have created a new software for annotation of the Blastocrithidia genome, as existing annotation programs are not able to deal with ambiguous stop codons. This allowed us to look at the reassigned stop codons across the whole genome, which may shed light on the general trends in their features and distribution. The ultimate goal of our study is to address the following intriguing questions: How does translation termination function without defined stop codons? How do numerous reassigned stop codons influence translation? What are the prerequisites of this lineage that made the reassignment possible? What could be the intermediate steps between a system with a standard genetic code and one with all three stop codons reassigned? I will try to answer some of these questions during my talk.

18 Cas9 editing for precision dissection of high priority anti-trypanosomal drug targets

Rico, Eva; Wall, Richard; Roberts, Adam; Lukac, Iva; Zuccotto, Fabio; Gilbert, Ian; Fairlamb, Alan; Wyllie, Susan; Horn, David (Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK)

Early-stage drug discovery relies upon promising, genetically validated targets, or active compounds identified through phenotypic screening. Unfortunately, genetically-validated targets are often not druggable, while the molecular targets of phenotypically-active compounds are not determined, hindering optimisation of these compounds. Target deconvolution can greatly facilitate drug discovery by yielding promising, chemically-validated targets for further development. This knowledge can be used to improve potency, reduce toxicity and understand resistance. Consequently, we have used integrated genetic and proteomic screening approaches for target deconvolution. CRISPR/Cas9-based editing has then been used to validate and dissect new high priority targets that have emerged from these studies. 1). Cleavage and polyadenylation specificity factor 3 (CPSF3) is the target of acoziborole, in advanced phase 2/3 clinical trials against sleeping sickness, and AN11736, in development against nagana. A docking model suggested a steric clash between acoziborole and the human enzyme, explaining specific anti-trypanosomal activity. Mutagenesis using a 64-fold degenerate template confirmed this hypothesis and revealed a specific Asn232His mutation in resistant parasites. 2). Cyclin-dependent kinase 12 (CRK12) has been confirmed as the molecular target of a pre-clinical candidate for visceral leishmaniasis and is a pharmacologically-validated target in T. brucei. In this case, Cas9 editing revealed several resistance-conferring mutations which were entirely consistent with our docking model. 3). The proteasome is a validated target in all three kinetoplastids. Here, inducible Cas9 was used to precision edit the Trypanosoma cruzi proteasome beta-4 subunit, again conferring drug resistance. Thus, precision editing provides robust target-validation, defines drug-resistant strains and allows for detailed target dissection. These new genetically/chemically/pharmacologically validated targets are now prioritised for target-based drug-discovery efforts.

19 Characterization of proline transporter encoded by the AAT7 gene family in Trypanosoma brucei

Haindrich, Alexander C.; Wirdnam, Corina; Niederhauser, Isabel; Rentsch, Doris (IPS, Univeristy of Bern, Bern, CHE)

Trypanosoma brucei faces a number of nutritional challenges during its life cycle. One major adaption is the utilization of proline for energy metabolism in the insect stage of T. brucei, in contrast to glucose-dependent energy production in blood stream form parasites and in early procyclics.

Proline metabolism and proline uptake have previously been studied in trypanosomatid parasites, but little was known on genes encoding proline transporters. While in Leishmania the amino acid transporter AAP24 seems the major proline uptake system, the homolog in T. brucei, AAT6, was shown to transport a variety of neutral amino acids in addition to proline. Furthermore, AAT6 loss did not affect growth.

We found that four members of the AAT7 gene family are able to mediate transport of proline, alanine and cysteine when expressed in Saccharomyces cerevisiae, including one high affinity proline transporter (Tb427.08.7610, Km 15 µM). Knock-down of the transporters by RNAi in procyclic form T. brucei resulted in a reduction of the intracellular proline concentration and was shown to be lethal during low proline availability under standard and glucose-free culture conditions.

RNAseq analyses further showed that transcript levels of these AAT7 genes respond to amino acid deprivation and glucose depletion with an increase of transcript abundance in procyclic form T. brucei. A similar albeit weaker response was found during amino acid starvation of bloodstream form parasites.

20 Characterizing the role of TbAMPK and TbTOR in nutrient signaling in the African trypanosome

Jones, Jessica A; Morris, James (Clemson University)

Trypanosoma brucei has a complex life cycle is that includes stages in the mammalian host and tsetse fly vector. Inhabiting these distinct niches requires adaptation through morphological and metabolic reprogramming to endure differences in available nutrients, temperature, and pH. In the mammalian blood, the proliferative long slender bloodstream form (LS) parasites primarily rely on glycolysis to generate ATP, whereas insect stage procyclic from (PF) parasites generate ATP through more diverse metabolic program. The developmental program includes the differentiation of the LS in the mammalian blood to a quiescent form preadapted for life in the fly, the short stumpy (SS) form, followed by SS differentiation to PF in the fly gut. While several cues for differentiation have been identified, it is unknown how these cues are integrated into a signal to activate differentiation. TOR proteins, which are known master regulators that respond to nutrient availability in other eukaryotes, are candidates for participating in this response. Additionally, AMP-dependent protein kinase (AMPK), a known regulator of cellular energy homeostasis, is a candidate to play a role in the nutrient-based transmission of differentiation cues. Here, the role of TbTOR4 and TbAMPK on differentiation will be assessed. Published work from others indicate that TbAMPK activity increases and TbTOR4 abundance decreases during parasite transition from LS to PF (Saldivia 2016). We have found that TbAMPK phosphorylation is regulated in response to environmental glucose. Further, while RNAi of TbTOR4 does not elicit a growth phenotype in PF parasites, silencing TbTOR4 leads to dysregulation of transcripts including citrate synthase and hexokinase in a manner that is dependent on environmental glucose availability. These observations support the hypothesis that TbAMPK and TbTOR4 play a role in the signaling cascade controlling T. brucei differentiation.

21 Conditional gene ablation with DiCre and CRISPR/Cas9 reveals the involvement of homologous recombination factors in DNA replication and genome variability in Leishmania

Damasceno, Jeziel; Reis-Cunha, Joao (Universidade Federal de Minas Gerais); Crouch, Kathryn (University of Glasgow); Bartholomeu, Danielle (Universidade Federal de Minas Gerais); McCulloch, Richard (University of Glasgow, Glasgow, United Kingdom)

While some homologous recombination (HR) factors have been implicated in gene amplification in Leishmania, the depth and breadth of HR roles in genome maintenance remains unexplored in this parasite. Due to the potentially unusual nature of DNA replication initiation in Leishmania, in which a single locus-specific origin is activated per chromosome, we set out to investigate the roles of HR in the replication process of this parasite. Using CRISPR/Cas9, we engineered five cell lines that allow DiCre-mediated inducible knockout (KO) of various HR genes (Rad51 and its paralogues - Rad51-3, Rad51-4, Rad51-6 – and the helicase PIF6). Except for PIF6, induced KO of the HR genes led to severe growth defects upon long term cultivation. In addition, loss of each Rad51-related gene resulted in accumulation of DNA damage, as measured by gammaH2A, demonstrating all these factors act in the preservation of genome integrity. Induced KO of Rad51 and Rad51-3 led to decreased proportions of S-phase cells, defective DNA synthesis, and increased gammaH2A levels during S-phase navigation. However, whole genome sequencing revealed that while Rad51 loss led to increased genome-wide instability, the opposite effect was seen upon Rad51-3 loss. Also, distinct patterns of genomic instability around regions of transcription initiation and/or termination was seen upon loss of each of these factors. Altogether, these data indicate: 1) Rad51 and Rad51-3 have a role during DNA replication in Leishmania; 2) they act through antagonist mechanisms; and 3) they might be involved in the resolution of replication/transcription conflicts. Currently, we are characterizing the genome-wide patterns of DNA replication upon induction of Rad51 and Rad51-3 KO.

22 Control of RNA editing fidelity in trypanosomes

Meehan, Joshua; Kumar, Vikas (Texas A&M University, Biochemistry); Gulati, Shelly (University of Oklahoma Health Sciences Center); Doharey, Pawan (Texas A&M University, Biochemistry); Ivens, Al (University of Edinburgh, Institute of Immunology); Mcdermott, Suzanne (Center for Infectious Disease Research); Schnaufer, Achim (University of Edinburgh, Institute of Immunology); Stuart, Ken (Center for Infectious Disease Research); Mooers, Blaine (University of Oklahoma Health Sciences Center); Cruz-Reyes, Jorge (Texas A&M University, Biochemistry)

Massive posttranscriptional insertion and deletion of uridylates (U-indels) remodel the mRNA transcriptome in the single mitochondrion of kinetoplastids. The holo-editosome machine with >40 proteins, includes several macromolecular mRNA trans-factors (i.e., the core enzyme and auxiliary complexes) and thousands of small antisense RNAs that direct editing. After the discovery of RNA editing in 1986 this major question is still outstanding: What are the key checkpoints and basic molecular mechanisms that control editing? We identified a trans-factor that includes a regulatory RNA helicase REH2, and an unusual zinc-finger protein H2F1. Our RNAseq analyses in isolated editosomes indicate that this helicase complex controls the fidelity of the U-indels, and the zinc-finger protein affects the helicase stability. We reconstituted an active helicase complex using full-length recombinant proteins, REH2 (242 kDa) and H2F1 (62 kDa), and began biochemical analyses of the recombinant vs native helicases. Also, we undertook a structural approach to examine movements of the helicase domains. Overall, we are using a multidisciplinary approach to study a novel regulatory helicase complex in RNA editing.

23 Controlling the surface proteome: A novel nndosomal tetratricopeptide-repeat protein interacts with deubiquitylase TbUsp7 and the TbSkp1-like protein in Trypanosoma brucei.

Yamada, Kayo Canavante, Ricardo; Zoltner, Martin; Field, Mark (University of Dundee)

S-phase kinase-associated protein 1 (SKP1) is plays a crucial role in cell cycle progression, transcriptional regulation, signal transduction, and many other cellular processes by virtue of being a component of Cullen E3 ubiquitin ligases. Phylogenetic analysis revealed that Trypanosoma brucei possesses at least three Skp-1/Elongin like proteins. Two deubiquitilating enzymes (DUBs), TbUsp7 and TbVdu1, control the abundance of the invariant surface proteins ISG75 and ISG65. ISG75 trafficking is key to suramin uptake and TbUsp7 silencing partially blocked endocytosis. Using untargeted proteomics, we find that the Skp1-like protein of trypanosomes, Tb927.10.11610, decreases in abundance following TbUsp7 knock down and physically interacts with TbUsp7 and a tetratricopeptide-repeat (TPR) protein (Tb927.11.810) which is located close to the flagellar pocket/endosomes, indicating a likely role in endocytosis. Tb927.11.810 is also implicated in suramin sensitivity. Further, silencing Tb927.10.11610 reduced the abundance of TbUsp7 and Tb927.11.810 TPR protein. Taken together these data suggest that Tb927.11.810 is a novel component of the endosomal system that has specific responsibilities for coordinating ubiquitylation of surface proteins.

24 Correlating Trypanosoma brucei localisation, tryptophan metabolism and behavioural changes in the rodent model of HAT

Fathallah N.A.1, Dawson N.1, Urbaniak M.D.1
1Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, United Kingdom;

Trypanosoma brucei is an extracellular protozoan parasite that causes Human African Sleeping Sickness (HAT). Trypanosomes are found in the blood, lymphatic system, adipose tissue,5 and skin1 during the early stage infection, and additionally in the brain in the late stage infection. The late stage is characterised by neuropsychiatric symptoms and sleep disorder that occur through an undefined mechanism.

Trypanosomes cultured in vitro significantly deplete tryptophan from the media, using it in both protein synthesis and transamination reactions.4 As well as forming an essential component of the kynurenine pathway, with both neurotoxic and neuroprotective branches, tryptophan is also the precursor of serotonin and melatonin which are implicated in sleep regulation.3 Research has also shown changes in brain tryptophan levels and increased 5-Hydroxytryptophan in stage II clinical samples.6

In this study, we are investigating the connection between parasite localisation, tryptophan metabolism, and behaviour in the murine model of late stage HAT. Our ex vivo imaging data using red-shifted luciferase expressing parasites2 reveals parasite sequestration to many organs during early stage infection. HPLC-based metabolomic analysis of tissue confirmed changes in tryptophan metabolism occur during the course of infection. Functional brain imaging and behavioural changes support altered brain function during late stage HAT. Taken together this data suggests a correlation between parasite localisation, tryptophan metabolism and behavioural changes that may be clinically relevant.

Capewell P., et al. (2016). The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. Elife 5: e17716

McLatchie AP., et al. (2013). Highly sensitive in vivo imaging of Trypanosoma brucei expressing “red-shifted” luciferase. PLoS neglected tropical diseases (11): e2571

Rodgers J., et al. (2009). Kynurenine pathway inhibition reduces central nervous system inflammation in a model of human African trypanosomiasis.Brain. 132(5): 1259-1267

Stibbs H.H., & Seed J.R., (1975) Short-term metabolism of [14C] tryptophan in rats infected with Trypanosoma brucei gambiense. Journal of Infectious Diseases. 131(4): 459-462

Trindade S., et al. (2016). Trypanosoma brucei parasites occupy and functionally adapt to the adipose tissue in mice. Cell host & microbe 19(6):837-848

Vincent IM., et al. (2016). Metabolomics identifies multiple candidate biomarkers to diagnose and stage human African trypanosomiasis. PLoS neglected tropical diseases

25 Cyclophilin 19 a new player in oxidative stress during Trypanosoma cruzi infection.

Pedroso dos Santos, Gregory Midori Abukawa, Fernanda (Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil); Maria Alcântara, Laura (Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil); Silvio Moretti, Nilmar (Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil); S. Mcgwire, Bradford (Division of Infectious Diseases/Center for Microbial Interface Biology, The Ohio State University); Schenkman, Sergio (Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil)

Cyclophilins are ubiquitous and evolutionarily conserved enzymes, known for its peptide-prolyl-cis-trans-isomerase activity. Several types of cyclophilins are expressed from prokaryotes to eukaryotes. Mammalian cyclophilin A (CyPA) is an intracellular enzyme that under stress situations has an increased expression, which also causes its secretion into extracellular environment. The extracellular CyPA (eCyPA) shows to play an important role during inflammatory processes such as cell migration, proliferation and T-cells activation. Inhibition of eCyPA expression and activity, decrease inflammation by preventing the recruitment of T-cells and reducing the production of metalloproteinase-9 (MMP-9) and IL-6. Trypanosoma cruzi, the protozoan parasite that causes Chagas disease, encodes the cyclophilin 19 (TcCyP19), which is very similar to CyPA. Here we found that TcCyP19 is expressed and distributed in the parasite cytosol of all lifecycle stages. Tissue culture trypomastigotes exposed to medium with acid pH or lacking glucose and serum, differentiated into amastigotes and secreted preferentially several cytosolic proteins, including TcCyP19 in the soluble form. By using TcCyP19 tagged with the HA epitope, we observed that intracellular amastigotes also release TcCyP19 in the host cell cytosol. We produced a lineage of myoblast L6 cells expressing the TcCyP19 and when measured the basal ROS levels, we found an increased amount of H2O2 production. When these cells were infected, the intracellular amastigotes showed an augmented replication profile as the same occurs in the infection with overexpressing TcCyP19 parasites in wild type L6 cells. Taking together, these results, suggest that TcCyP19 released by mammalian stages of T. cruzi could have a similar role as CyPA and affect the pathogenesis of Chagas disease.

26 Deciphering the molecular machinery of the antigenic variation regulator DOT1B

Eisenhuth, Nicole; Vellmer, Tim (Department of Cell and Developmental Biology, Würzburg, Germany); Dejung, Mario; Butter, Falk (Institute of Molecular Biology, Mainz, Germany); Janzen, Christian J (Department of Cell and Developmental Biology, Würzburg, Germany)

Antigenic variation is an essential mechanism for survival of the protist parasite Trypanosoma brucei inside its mammalian host. This process is mediated by tightly controlled monoallelic expression of Variant Surface Glycoproteins (VSG) from one of 15 subtelomeric VSG expression sites (ES).

It has been shown previously that the conserved histone methyltransferase DOT1B is involved in ES regulation. DOT1B knockout cells show derepression of transcriptionally silent telomeric VSGs and extremely slow ES in situ switch kinetics. Furthermore, ES silencing during differentiation from the mammalian-infective stage to the insect-infective stage is also slower compared to wild-type cells. Finally, the attenuation of the active ES in response to inducible expression of an additional VSG gene is not observed in DOT1B negative parasites. The molecular machinery, which enables DOT1B to execute regulatory functions at the active ES is still elusive, however.

To learn more about DOT1B-mediated processes, we purified DOT1B protein complexes using complementary biochemical approaches. Through a combination of tandem affinity purification and proximity-dependent biotin identification (BioID), we identified several DOT1B-interacting proteins. Surprisingly, one of the most abundant DOT1B-associated protein complexes was RNase H2, which resolves DNA/RNA hybrids and is important for the maintenance of genome integrity.

Since DNA double strand breaks (DSBs) in the active ES lead to a recombination-based VSG switch, we monitored DSB formation in DOT1B-depleted cells. Although DOT1B depletion caused an initial accumulation of DSBs, this phenotype was attenuated after several generations. Proteomic data provide insight into the underlying adaptation process. A novel putative mechanism of ES regulation will be discussed.

27 Deep mutational scanning reveals developmentally distinct functions for KREPB4 in RNA editing

McDermott, Suzanne M; Stuart, Kenneth (Seattle Children's Research Institute)

Uridine insertion and deletion RNA editing generates functional mitochondrial mRNAs in T. brucei. Several transcripts are differentially edited via unknown mechanisms in bloodstream (BF) and procyclic (PF) forms, correlating with differences in mitochondrial function. Editing is catalyzed by three ~20S editosomes that have several proteins in common, including enzymes that add or remove Us or ligate RNA, but are distinguished by different RNase III endonuclease/partner protein sets and distinct editing site preferences. Several common editosome proteins, including KREPB4, lack homology to known catalytic domains and their precise functions are unclear. KREPB4 has zinc-finger and PUF motifs and, like several other editosome proteins, a divergent, likely noncatalytic RNase III domain. We show that KREPB4 is essential for BF and PF growth, editing, and editosome integrity, but its loss has differential effects on editosome components and complexes in BF and PF. We developed a high-throughput deep mutational scanning approach to assess the effects of many amino acid substitutions on KREPB4 function in BF and PF with an initial focus on its divergent RNase III domain. Deep mutational scanning of this domain in both life-cycle stages identified many single amino acid substitutions that had various phenotypic effects. These included severe disruption of growth, editing, and editosomes in both BF and PF, as well as differential effects on BF and PF cell growth. We also showed that the KREPB4 RNase III domain is required for the association with editosomes of all editing endonucleases, consistent with cross-linking mass-spectrometry analyses of KREPB4 interactions. Our results support a central role for the KREPB4 RNase III domain in editing, and its involvement in regulation of editing during the T. brucei life-cycle. They indicate that substrate recognition and cleavage by editing endonucleases may be a key developmental regulatory point in editing. They also illustrate the power of this high-throughput method to study sequence-function relationships in proteins that lack homology to known domains.

28 Different extracellular vesicles are secreted from Trypanosoma brucei under starvation, heat shock and drugs-biochemical and functional analyses

Michaeli, Shulamit; Kennan, Sriram; Eliaz, Dror (Bar-Ilan University)

In Trypnosoma bruceiinhibition of trans-splicing either by depletion of essential splicing factors or heat shock results in export of the spliced leader RNA (SL RNA) to the cytoplasm and then its secretion by exosomes. The exosomes are formed in multivesicular bodies (MVB) utilizing the endosomal sorting complexes required for transport (ESCRT) but not vesicles that are derived from nanotubes associated with the flagella. These exosomes affect social motility (SoMo) (Eliaz et al., PLoS pathogens 2017). Exosomes secreted from Leishmania species under heat-shock affect the motility of T. brucei indicating the presence of interspecies signals in these exosomes. The exosomes were purified and their proteome and RNome was determined. Factors common to exosomes derived from other trypanosomatid species were identified but also factors implicated in social motility. The small RNome identified RNAs involved in SL RNA biogenesis and certain half-tRNAs. Interestingly, trypanosomes subjected to starvation secrete vesicles that differ from these exosomes. The starvation induced vesicles (SIV) are shed from the posterior part of the parasite, lack SL RNA and have no effect of social motility, suggesting that SL RNA secretion is correlated with the effect on motility. Trypanosomes sense and “escape” from exosomes containing SL RNA but can not sense or avoid the migration towards dangerous compounds such as nanoparticles bound by a lytic polymer that lyse the parasites. The parasite population was unable to sense the drug and “escape” from it. The biological function of the different vesicles secreted under different cues will be discussed.

29 Discovery of a kinetochore-based ATM/ATR-independent DNA damage checkpoint in Trypanosoma brucei

Zhou, Qing Pham, Kieu T. M.; Hu, Huiqing; Kurasawa, Yasuhiro; Li, Ziyin (University of Texas Health Science Center)

To maintain genome integrity in eukaryotes, DNA damage-induced G2/M cell cycle checkpoints arrest the cell cycle, allowing cells to repair damaged DNA before entering mitosis. The signaling pathway for this cell cycle checkpoint involves the ataxia telangiectasia mutated serine-protein kinase (ATM) and ataxia telangiectasia and Rad3 related protein kinase (ATR), which phosphorylates and activates the cell cycle checkpoint kinase Chk1 for the latter to inhibit the Cdc25 phosphatase, thereby leading to inactivation of the G2/M-phase cyclin-dependent kinase Cdk1. This G2/M checkpoint pathway is evolutionarily conserved from yeast to humans, but Trypanosoma brucei appears to lack Chk1 and Cdc25 homologs, suggesting the existence of a novel DNA damage-induced G2/M checkpoint pathway. Here, we report the discovery of a kinetochore-based, ATM/ATR-independent DNA damage-induced G2/M cell cycle checkpoint in T. brucei. We found that MMS-induced DNA damage triggers a G2/M cell cycle arrest by stabilizing the mitotic cyclin CYC6 and the cohesin subunit SCC1, and that depletion of ATM and ATR does not lead to a bypass of the DNA damage-induced G2/M cell cycle arrest. We further found that DNA damage modulates the abundance of the outer kinetochore protein KKIP5, in an ATM/ATR-independent, but Aurora B kinase- and kinetochore-dependent manner, suggesting a role of KKIP5 in DNA damage response. Consistent with this, overexpression of KKIP5 arrests cells at the G2/M boundary through stabilizing CYC6 and SCC1, mimicking DNA damage-induced G2/M arrest, whereas depletion of KKIP5 alleviates the DNA damage-induced G2/M arrest and causes chromosome mis-segregation and aneuploidy. These findings suggest that trypanosomes employ an unusual DNA damage-induced G2/M checkpoint to maintain genomic integrity.

30 Divergent RNase III domains in KREPB6, KREPB7, and KREPB8 are essential for editing in both procyclic and bloodstream form Trypanosoma brucei

Carnes, Jason; McDermott, Suzanne; Stuart, Kenneth (Seattle Children's Research Institute)

The ~20S multiprotein editosome complexes catalyze the gRNA specified insertion and deletion of uridines to create mature mitochondrial mRNAs in Trypanosoma brucei. Three functionally distinct editosomes are distinguished by their single KREN1, KREN2, or KREN3 RNase III endonuclease and, respectively, the KREPB8, KREPB7, and KREPB6 partner proteins. The endonucleases perform the first catalytic step of editing, precisely cleaving mRNA in the vastly diverse mRNA/gRNA heteroduplex substrates. We previously identified divergent, likely non-catalytic RNase III domains in the KREPB6, KREPB7, and KREPB8 partner proteins, as well as in editosome proteins KREPB4, KREPB5, KREPB9, and KREPB10. Because known RNase III endonucleases function as dimers, the editing endonucleases may form catalytically active heterodimers with one or more of these non-catalytic RNase III proteins. We show here by analysis of conditional null cell lines that KREPB6, KREPB7, and KREPB8 are essential in both procyclic form (PF) and bloodstream (BF) cells. Loss of these proteins results in growth defects and loss of editing in vivo, as does a mutation to the RNase III domain that is predicted to prevent dimerization. These results suggest that KREPB6, KREPB7, and KREPB8 form heterodimers with their respective endonucleases that provide for specific cleavage of the mRNA in heteroduplex mRNA/gRNA substrates. The impact of the loss of KREPB6, KREPB7, or KREPB8 on partner endonuclease abundance and RNA editing differs dramatically between life cycle stages, with greater defects observed in BF relative to PF. This implies that developmental regulation of editing may include control of endonuclease function. We also present a model wherein other editosome proteins with divergent RNase III domains function in substrate selection via enzyme-pseudoenzyme dynamics.

31 Editing of Minimally Edited RNAs in Trypanosoma brucei

Tylec, Brianna L.; Simpson, Rachel; Chen, Runpu; Sun, Yijun; Read, Laurie (University at Buffalo)

Uridine (U) insertion/deletion editing of select mitochondrial mRNAs is an essential process in kinetoplastids whereby mRNA is modified to generate a translatable open reading frame with the use of trans-acting guide RNAs (gRNAs). gRNAs are sequentially utilized as templates, which ensures the general 3' to 5' progression of editing along the mRNA. This process involves multiple protein subcomplexes including the non-enzymatic RNA Editing Substrate Binding Complex (RESC), composed of the RNA Editing Mediator Complex (REMC) and Guide RNA Binding Complex (GRBC). Nine mitochondrial RNAs are edited throughout the length of the transcript, termed "pan-edited", while three genes only require editing over a small portion of the reading frame. These "minimally edited" RNAs are a good model for studying editing since they require only 1-2 gRNAs to complete canonical editing. We characterized the editing of the minimally edited RNA, CYb, in procyclic form T. brucei using our previously published Trypanosome RNA Editing Alignment Tool (TREAT). We found that minimally edited transcripts face barriers to editing distinct from those primarily affecting pan-edited mRNAs. We also demonstrate that mis-edited regions of partially edited transcripts termed junctions can form via multiple mechanisms including mis-alignment of cognate gRNA, incorrect gRNA utilization and inefficient gRNA anchoring. In addition, we evaluated the roles of editing accessory factors MRP1/2 and RBP16 via RNAi and saw that they affect the editing of CYb though different mechanisms. RBP16 primarily affects the initiation of CYb editing as well as the progression of editing, while MRP1/2 appears to stabilize partially edited mRNAs.

32 Effects of small molecule inhibitors of DNA break repair on Trypanosoma brucei

Hovel-Miner, Galadriel; Wallin, Stephen; Howard, J. Nataile (George Washington University)

Trypanosoma bruceiis an early branching eukaryote whose DNA break repair pathways demonstrate both conserved and divergent features with humans and well-studied model organisms. Members of the genus kinetoplastida appear to lack canonical non-homologous end-joining (NHEJ) for DNA double-stranded break (DSB) repair. In these parasites DSB are predominantly repaired by homologous recombination (HR) and, to a lesser extent, by microhomology-mediated end-joining. Following RAD51 loading onto broken ssDNA filaments, with assistance from BRCA2, little is known about the subsequent steps of HR in T. brucei. While T. brucei has an MRE11 homolog, it is not required for homologous recombination events associated with antigenic variation, which are largely dependent on RAD51. Elucidation of DNA break repair pathways by traditional genetic methods is hindered by the availability of sufficient selectable markers to generate and characterize multiple genetic knock-outs. Chemical inhibition of specific steps of DNA DSB repair has emerged as a novel therapeutic avenue in cancer treatment, therefore we explored the usefulness of chemical inhibition of DNA break repair in T. brucei. For this study, we utilized the RAD51 inhibitor RI-1 and the MRE11 inhibitor mirin, whose functional bindings sites are conserved in their T. bruceihomologs. We found that RI-1 largely recapitulated phenotypes associated with TbRAD51 knockout studies, including increased sensitivity to the DNA damaging agent Methyl methanesulfonate (MMS). Treatment with mirin showed similar effects seen in MRE11 knockouts on cell growth and survival post DNA damage, but also reduced cell cycle defects and overall DNA damage accumulation associated with MMS. Thus, small molecule inhibitors of DNA break repair pathways can provide an important new analytical tool for the for the dissection of DNA break repair pathways in kinetoplastida. Elucidating the DNA break repair pathways of trypanosome parasites is significant because: African trypanosome antigenic variation is rooted in DNA break repair, identification of unique aspects of kinetoplastida DNA break repair could result in anti-parasitic drugs, and identification of non-canonical DSB repair pathways might provide new insights into human cancers and genetic disorders.

33 END-seq: a new whole-genome approach for the analysis of DNA double-stranded breaks in the African Trypanosome

Sciascia, Nicholas Quinn, McKenzie (George Washington University); Wu, Wei (National Cancer Institute, NIH); Hovel-Miner, Galadriel (George Washington University)

African trypanosomes are able to change their antigenic surface through recombination events that translocate a silent Variant Surface Glycoprotein (VSG) gene into an actively transcribed site. These recombination events are expected to originate with the formation of a DNA break, which is likely to be double-stranded. In addition VSGs undergo recombination events that result in mosaicisms that form new and antigenically distinct VSGs. However, the sites and sources of DNA breaks that promote either VSG switching by recombination or mosaic VSG formation are unknown. END-seq is a whole-genome sequencing approach for mapping DNA double-stranded breaks (DSB) genome wide and has been well established for immune cells in mammalian systems. END-seq utilizes a specific biotinylated hairpin adaptor to bind DSBs (suspended in a agarose matrix) for their capture and subsequent next-generation sequencing. Here we have conducted preliminary experiments to assess DBS formation across the T. bruceiLister 427 genome, the VSG Expression Sites (ES), and during the induction of a DSB in the active VSG expression site. The application of a standard END-seq protocol produced robust results for the T. bruceiLister 427 genome, enabling DSB to be mapped both genome wide and in VSG ESs. Preliminary analysis of DSB distributions will be presented as well as the effects of ES induced break formation. These data suggest that, with additional controls and experimental refinement, END-seq is a new and powerful tool at our disposal for better understanding the effect of DSB formation on the T. bruceigenome including its potential effects on VSG switching.

34 Epigenetic regulation at replicative and non-replicative forms of Trypanosoma cruzi

P.C. da Cunha, Julia Poubel, Saloe Bispo (Butantan Institute); Lima, Alex Ranieri Jerônimo (UFPA); Roson, Juliana Nunes; Araujo, Christiane (Butantan Institute); Gonçalves, Evonildo (UFPA); Masotti, Cibele; A.F. Galante, Pedro (Sirio Libanes Hospital); Elias, Maria Carolina (Butantan Institute)

Trypanosoma cruzi alternates between replicative and non-replicative forms accompanied by a shift on global transcription levels and by changes in its chromatin proteome and architecture. To gain insights about epigenetics regulation that may guide life forms differences, we performed genome-wide and proteomics approaches using replicative and non-replicative T.cuzi forms. Remarkly, both MNase and FAIRE-seq studies indicate that epigenome reflect phenotypic biological differences observed between life forms. Genomic regions associated with DNA replication are enriched in well-positioned nucleosomes mainly at the non-replicative forms (trypomastigotes) while multi-family genes – mainly associated with infective-stage and virulence factors, are over represented at non-infective forms. The putative RNA Pol II transcriptional start sites (TSS) and termination sites (TTS) from epimastigotes and trypomastigotes differ both regard amount/distribution of well-positioned nucleosomes and/or active chromatin regions. Whether it is cause or consequence of the global transcription rate differences found between life forms, needs to be further evaluated. Previously, we found that chromatin from non-replicative forms are enriched of a histone H2B variant (H2Bv), which are mainly located at T.brucei TSS. To better explore T.cruzi TSS regulation, we performed pulldown assays to investigate the interaction partners of H2Bv. Interestingly, this variant interacts preferentially, at non-replicative stages, with the bromodomain factor-2 suggesting that a histone acetylation specific pattern may occur at TSS from non-replicative forms. Taken together, our results pointed out that gene/proteomic expression may be, indeed, a reflection of epigenetic changes even in a context with lack of canonical transcriptional control.

35 Evolution of metabolic and molecular features in Kinetoplastea revealed by comparative genomics

Butenko, Anzhelika Flegontova, Olga; Horák, Aleš (Institute of Parasitology, Biology Centre, Ceské Budejovice, Czech Republic); Hampl, Vladimír (Faculty of Science, Charles University, Prague, Czech Republic,); Keeling, Patrick (Department of Botany, University of British Columbia, Vancouver, Canada,); Gawryluk, Ryan (Department of Biology, University of Victoria, Victoria, Canada,); Tikhonenkov, Denis (Department of Botany, University of British Columbia, Vancouver, Canada; Institute for Biology of Inland Waters, Russian Academy of Sciences,); Flegontov, Pavel (Institute of Parasitology, Biology Centre, Ceské Budejovice, Czech Republic; Faculty of Science, University of Ostrava, Ostrava, Czech Republic,); Lukeš, Julius (Institute of Parasitology, Biology Centre, Ceské Budejovice; Faculty of Science, University of South Bohemia, Ceské Budejovice, Czech Republic,)

Kinetoplastea is a group of protists with tremendously different lifestyles, comprising two major clades, Prokinetoplastida and Metakinetoplastida. The latter is subdivided into four groups: i) invariably parasitic Trypanosomatidae; ii) eubodonids, their closest free-living relatives; iii) primarily free-living and commensal neobodonids, with a single known parasitic species Azumiobodo, and iv) parabodonids, uniting free-living and parasitic members of the genera Parabodo, Trypanoplasma, etc. Prokinetolastids represent the most basal branching kinetoplastid clade with well-described members of the genera Ichthyobodo, Perkinsela and uncultured representatives from environmental samples. The closest free-living relatives of kinetoplastids are diplonemids, possibly the most diverse marine planktonic eukaryotes, and euglenids, with best known photosynthetic representatives, which together with bacteria-covered postgaardians comprise the phylum Euglenozoa. The family Trypanosomatidae, which incorporates Trypanosoma and Leishmania spp. is by far the most studied group. The availability of sequencing data for euglenozoans is a prerequisite for understanding their evolution. Yet only fragmentary information is available for species outside Trypanosomatidae.

We have sequenced the transcriptomes of three diplonemids, three kinetoplastids (including two free-living prokinetoplastids) and two euglenids and combined our data with 10 publicly available genomes and transcriptomes. The genome-wide comparative analysis of metabolism revealed that trypanosomatids and bodonids, except for free-living prokinetoplastids, are characterized by lower metabolic capabilities compared to diplonemids and euglenids. Important enzymes of amino acid, vitamins and cofactors' biosynthesis, and nucleotide metabolism were lost in all kinetoplastids or gradually within the kinetoplastid tree. Using diplonemids as an outgroup allowed us to study the evolution of the origin recognition complex, thiol redox systems and kinetochores in Euglenozoa. The latter appear to be highly divergent in diplonemids and, yet, different from the unconventional kinetoplastid machinery.

36 Flow Cytometry-Assisted Fluorescent Barcoding for Simultaneous Interrogation of Multiple Metabolites in Live Kinetoplastid Parasites

Vance, Jacob Voyton, Charles (Clemson University); Christensen, Kenneth A. (Brigham Young University, Provo, UT)

Human African Trypanosomiasis (HAT), caused by the single-celled protozoan Trypanosoma brucei, is a pressing public health issue in sub-Saharan Africa. T brucei glucose metabolism is an attractive therapeutic target, but a lack of in vivo analytical approaches for measuring key metabolites in intact parasites has limited progress in identifying compounds that target glucose metabolism. Recently, we have used endogenously expressed fluorescence resonance energy transfer (FRET) biosensors that measure intracellular and intraglycosomal glucose to perform high-throughput screening for glucose uptake inhibitors. We have also used sensor-expressing trypanosomes to track the dynamics of intracellular glucose under changing extracellular conditions. However, monitoring only a single analyte limits our ability to study a complex process such as metabolism, which has multiple interdependent analytes, including glucose, ATP, pH, and pyruvate. Importantly, the ability to monitor the dynamics of multiple metabolites in the same experiment, with high temporal resolution, should allow validation of current models of glycolysis in T. brucei. Here we demonstrate a cellular barcoding method, in which multiple cell lines expressing different metabolically relevant FRET biosensors are mixed and then analyzed together via flow cytometry. Each cell line is distinguished from one another based on a designed surface-fluorescence staining scheme, or barcode. With two surface-reactive fluorescent dyes, up to 9 unique populations can be analyzed in one experiment. Biosensor response is unaffected by barcoding. Hence, barcoding can be used to monitor multiple important analytes under a single set of conditions in a single experiment.

37 Functional interplays between Polo-like kinase and cytokinesis regulatory proteins in Trypanosoma brucei

Lee, Kyu j. Kurasawa, Yasuhiro; An, Tai; Li, Ziyin (University of Texas)

Trypanosoma brucei undergoes cytokinesis in a uni-directional manner along the longitudinal axis of the cell from the cell anterior towards the cell posterior, and is regulated by a novel signaling cascade consisting of two evolutionarily conserved protein kinases, the Polo-like kinase TbPLK and the Aurora B kinase TbAUK1, the kinetoplastid-specific protein phosphatase KPP1, and a cohort of trypanosome-specific regulators, including CIF1, CIF2 and CIF3. Our pevious work demonstrated that TbPLK functions as an upstream regulator to recruit these cytokinesis regulators to the cytokinesis initiation site, but mechanistically how TbPLK executes this function remains poorly understood. To better understand the interplays between TbPLK and other cytokinesis regulators, we carried out biochemical and genetic analyses. Through biochemical and mass spectrometry assays, we uncovered the structural motifs required for the interaction of TbPLK with CIF1, CIF2 and KPP1, identified CIF1 and CIF2 as TbPLK substrates, and determined the TbPLK phosphosites in CIF1 and CIF2. We showed that the N-terminal domain (NTD) and the intrinsically disordered region (IDR) of CIF1 mediate the interaction with TbPLK, and that deletion of NTD or IDR disrupted CIF1 function, impaired TbPLK localization and inhibited cytokinesis initiation. We mapped the TbPLK-interaction domain and the TbPLK phosphosites to the C-terminus of CIF2, and demonstrated that mutation of these phosphosites impaired CIF2 function and inhibited cytokinesis initiation. We identified distinct sub-domains in TbPLK for interaction with the Plus3 domain and the catalytic domain of KPP1, and uncovered a phosphorylation-dependent mechanism for the functional interplay between TbPLK and KPP1. Altogether, these findings provided novel insights into the mechanistic understanding of the TbPLK-mediated control of cytokinesis initiation in T. brucei.

38 Functionally mapping the evolutionary diversification of Trypanosoma congolense and Trypanosoma brucei using spatial proteomics

Moloney, Nicola Barylyuk, Konstantin; Lilley, Kathryn; Waller, Ross; MacGregor, Paula (University of Cambridge)

Protein function is often intimately linked with localisation and as a consequence the subcellular distribution of a protein can provide information on its role within the cell. We are optimising an effective isolation and separation method for achieving subcellular resolution of organelles in African trypanosomes for implementation in the proteomics-based strategy of hyperLOPIT (hyperplexed localisation of organelle proteins by isotope tagging). By coupling subcellular fractionation with multiplexed quantitative proteomics, protein abundance can be determined across multiple subcellular compartments simultaneously thereby generating a spatial proteome map. Following this, unknown proteins can be localised based on the distribution profiles of known organelle marker proteins using multivariate statistics and machine learning.

This work will provide a robust global spatial map of the Trypanosoma brucei and Trypanosoma congolense proteomes. Individually, these data sets will be informative towards the determination of uncharacterised protein functions, particularly for Trypanosoma congolense, where high-throughput functional analysis lags behind that of Trypanosoma brucei. Further, and more importantly, comparative analysis of these spatial maps will yield significant insight into the evolutionary diversification of these African trypanosome species and the effects of species specialisation on the molecular biology of the parasite cell.

39 Glycosome heterogeneity in Trypanosoma brucei is likely a function of organelle maturation and functional specialization

Crowe, Logan Wilkinson, Christina; Knight, Emily; Morris, Meredith (Clemson University)

Kinetoplastids have essential organelles called glycosomes that harbor numerous biochemical pathways. Multiple studies reveal that glycosomes are heterogeneous, however, the biological significance of this organelle diversity and the molecular mechanisms responsible for this variation and specialization are unknown. Previous work revealed that the glycosomal enzyme arginosuccinate localized to a subset of glycosomes (Lakhal-Naouar et al.) and super-resolution microscopy from our lab suggests that fructose 1,6-bisphosphatase (FBPase), and phosphofructokinase (PFK), involved in gluconeogenesis and glycolysis, respectively, exhibit different localization patterns. Compartment specialization requires that different locations be distinctly marked. The post-translational import of glycosome matrix proteins involves the docking and translocation machinery (DTM) comprised of glycosome membrane proteins and provides a potential mechanism for organelle specialization. In higher eukaryotes, Pex13 and Pex14 comprise the DTM of peroxisomes; organelles that are evolutionarily related to glycosomes. Kinetoplastids are unique in that they have two Pex13, Pex13.1 and Pex13.2, and we hypothesize that these proteins form complexes that mediate selective import of glycosome matrix proteins. We are using a number of biochemical techniques to define the DTM in T. brucei and are developing a flow cytometry-based method to define the protein composition of different glycosome populations. Our data suggests that glycosome specialization is likely the result of two processes; the maturation of pre-glycosomal vesicles into mature glycosomes and sequestration of different biochemical pathways to discrete organelles (functional specialization). We believe this work will reveal the extent to which glycosomes become specialized, provide insight into the mechanisms that contribute to glycosome heterogeneity, and inform our understanding of the biological significance of such variation.

40 Identification and characterization of the early acting glycosome biogenic protein PEX3 of Trypanosoma brucei- a target for drug development against the trypanosomatid diseases

Banerjee, Hiren Knoblach, Barbara; Rachubinski, Richard A. (University of Alberta)

Trypanosomatid parasites, including Trypanosoma and Leishmania, are the infectious zoonotic agents for a number of severe diseases like sleeping sickness and American trypanosomiasis (Chagas disease) that affect millions of people worldwide, mostly in the developing world. The glycosome is a specialized member of the peroxisome family of organelles that compartmentalizes normally cytosolic enzymes of the glycolytic pathway in these organisms. Glycosomes are essential for trypanosomatid viability, making them a prime target for drugs to kill these organisms by interfering with either their biochemical functions or their formation. Like other members of the peroxisome family, glycosome biogenesis is controlled by proteins called peroxins (PEX). The PEX3 protein is the master regulator of peroxisome biogenesis, and compromised PEX3 function results in cells devoid of peroxisomes and even lacking empty peroxisomal structures called 'peroxisomal ghosts'. Although PEX3 has been found throughout the diversity of eukaryotes, its identification in the trypanosomatids has been elusive. We used a bioinformatics approach to mine the proteins encoded by the Trypanosoma genomes and have now identified the trypanosomal PEX3 protein. Microscopic analysis and subcellular fractionation showed T. brucei PEX3 to be localized primarily to glycosomes. The interaction of PEX3 with the peroxisomal membrane protein receptor PEX19 observed for other eukaryotes is replicated by T. brucei PEX3 and PEX19, and mutations of conserved amino acids that abolish the interaction between PEX3 and PEX19 from other organisms also abolish the interaction of T. brucei PEX3 and PEX19. RNAi-mediated depletion of PEX3 in both the bloodstream and procyclic forms of T. brucei leads to mislocalization of glycosomal proteins to the cytosol, pronounced reduction in glycosome number, and cell death. The requirement for PEX3 for trypanosomal viability, combined with distinct differences in sequence between trypanosomal PEX3 and human PEX3, makes trypanosomal PEX3 a valuable target for drug development to combat diseases caused by the trypanosomatids.

41 IDENTIFYING CRITICAL INTERACTIONS IN THE UNIQUE TRYPANOSOMA BRUCEI 5S RIBONUCLEOPROTEIN COMPLEX AND THEIR ROLE IN RIBOSOME BIOGENESIS

Jaremko, Daniel Ciganda, Martin; Williams, Noreen (University at Buffalo, Jacobs School of Medicine and Biomedical Sciences)

Ribosome biogenesis is the process of assembling protein and RNA components into ribosomes. One critical regulatory checkpoint of this process is the formation and incorporation of the 5S ribonucleoprotein (RNP) into developing 60S ribosomal subunits. Work in our laboratory has identified the trypanosome-specific proteins P34/P37 as an essential part of the T. brucei nuclear 5S RNP. We have also shown direct in vitro interactions between P34/P37, L5, Rpf2 and 5S rRNA, components of the 5S RNP. Furthermore, we have identified unique properties of the T. brucei L5 and Rpf2 proteins that set them apart from their homologues in yeast.

We recently identified homologues of two other 5S RNP proteins, L11 and Rrs1, and used RNA interference to show that they are essential in T. brucei and play an important role in ribosome biogenesis. We then expressed recombinant L11 and used it to identify novel direct interactions with trypanosome-specific P34/P37 and 5S rRNA. These interactions highlight trypanosome-specific characteristics in a protein that is otherwise highly conserved across diverse organisms. We also expressed recombinant Rrs1 individually and as the Rpf2/Rrs1 complex, and showed that it independently interacts with 5S rRNA, an interaction that has not been observed in any other system.

Taken together, we have shown that L11 and Rrs1 are critical members of the 5S RNP in T. brucei. Furthermore, these proteins have unique properties as compared to yeast, highlighting the importance of studying key biological processes outside the context of model organisms. Ultimately, by mapping this network of interactions that occurs in the unique context of the T. brucei 5S RNP, we can identify targets for future drug development.

42 Insight into the active purinome of Leishmania donovani: Identification of potential novel purine-binding proteins

Leclercq, Olivier Dingli, Florent (Institut Curie, Laboratoire de spectrométrie de masse protéomique, Paris); Criscuolo, Alexis (Institut Pasteur, Centre de bioinformatique, bio-statistique et biologie intégrative, Paris); Guglielmini, Julien (Institut Pasteur, Centre de bioinformatique, bio-statistique et biologie intégrative, Par); Arras, Guillaume; Loew, Damarys (Institut Curie, Laboratoire de spectrométrie de masse protéomique, Paris); Rachidi, Najma; Späth, Gerald (Institut Pasteur and INSERM U1201, unité de parasitologie moléculaire et signalisation, Paris)

ATP-mediated signal transduction plays an important role in eukaryotic development and environmental adaptation. To decipher ATP-dependent signaling mechanisms involved in Leishmania survival, we performed a functional proteomic analysis based on ATP-affinity chromatography to capture all the functional ATP-binding proteins present in promastigote and axenic amastigote. First, we performed successive purification steps to capture low abundance proteins. Second, we insured that all the active proteins were captured by following the loss of ATP incorporation in the flow through. We identified 1064 putative purine-binding proteins (purinome), including 81 kinases. Most of the captured proteins were present in promastigotes and axenic amastigotes but some were specific of each stage, including 8 and 13 kinases, respectively. We also obtained 188 hypothetical proteins that were not previously identified as purine-binding proteins, suggesting that either they bind indirectly through interactions with purine-binding proteins, or directly through yet uncharacterized, parasite-specific domains. Bioinformatics analysis allowed us to determine protein motifs specific for each known purine-binding protein class, such as kinases, and ask whether the hypothetical proteins identified in our purinome also contained these motifs. We successfully classified 153 hypothetical proteins in 11 purine-binding protein classes. In particular, we identified 12 hypothetical proteins containing all 9 motifs specific of Leishmania kinases, suggesting that these proteins are potentially new members. Interestingly, most of them show a molecular weight above 100 KDa, and thus could be moonlighting proteins. We are currently producing recombinant proteins to assess whether they directly binds ATP. Our results establish the first purinome of any trypanosomatid, and allowed us to develop a novel approach combining affinity enrichment, proteomics and bioinformatics to annotate the Leishmania hypothetical proteome.

43 Interferon-gamma mediates inhibition of Plasmodium hepatic infection by Trypanosoma brucei

Sanches-Vaz, Margarida Luis, Rafael; Temporão, Adriana; Mendes, António M.; Goellner, Sarah; Carvalho, Tânia; Prudêncio, Miguel; Figueiredo, Luisa M. (Instituto de Medicina Molecular)

Sleeping sickness and malaria are two parasitic diseases with overlapping geographical distributions in sub-Saharan Africa. Therefore, these two parasites have likely been co-evolving in the same host and, as such, it is possible that they have developed strategies to compete with each other.

In order to understand if an ongoing T. brucei infection impacts a subsequent P. berghei liver infection, we established a co-infection model in which C57Bl/6J mice were initially infected by T. brucei, followed by administration of P. berghei sporozoites. We observed that when P. berghei sporozoites are inoculated into mice with an established T. brucei infection and detectable T. brucei parasitemia, the ensuing liver infection by the malaria parasite is ~90% lower than that observed in mice inoculated with P. berghei in the absence of a T. brucei infection. Furthermore, co-infected mice do not display severe malaria and survive longer. We further showed that the Trypanosoma inhibitory effect is very rapid: a strong decrease in the number of infected hepatocytes is detected as early as 30 min post-P. berghei sporozoite injection. Importantly, when we employed the co-infection protocol using RAG2- and IFN-γ-deficient mice, we found that, in contrast to wild-type mice, the P. berghei liver burden of RAG2- and IFN-γ-deficient co-infected mice was similar to that observed in mice inoculated only with Plasmodium. Hence, suggesting that lymphocyte-derived IFN-γ is required to impair the establishment of a subsequent P. berghei liver infection by T. brucei.

We have uncovered a novel inter-pathogen interaction whereby a primary infection by African Trypanosomes leads to the reduction of a subsequent liver infection by the malaria parasite and to an increase in host survival.

44 Loss of the haptoglobin-haemoglobin receptor in Trypanosoma brucei blocks life-cycle differentiation

Horakova, Eva Lacordier, Laurence; Cahuna, Paula (Laboratory of Molecular Parasitology, Institut de Biologie et de Medecince Moleculaires, Universite Libre de Bruxelles, B6041 Gosselies, Belgium); Changmai, Piya (Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceské Budejovice, Czech Republic); Sobotka, Roman (Institute of Microbiology, Czech Academy of Sciences, Trebon, Czech Republic); Lukeš, Julius (Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceské Budejovice, Czech Republic); Vanhollebeke, Benoit (Laboratory of Molecular Parasitology, Institut de Biologie et de Medecince Moleculaires, Universite Libre de Bruxelles, B6041 Gosselies, Belgium)

Trypanosomes are important parasites of humans and livestock. In their blood, they undergo extensive cellular differentiation, from dividing long slender to quiescent short stumpy forms. Due to the downregulation of the haptoglobin-haemoglobin receptor (HpHbR), uptake of the haptoglobin-haemoglobin complex is highly reduced in the stumpy forms. A single mutation in HbHpR unique to the human-infectious T. brucei gambiense is sufficient to dramatically reduce the binding and uptake of HpHb, leading to a negligible cellular haem level. The same phenotype was mimicked in T. b. brucei HpHbR knock out. For monitoring heme-dependent enzymatic activity, human catalase was expressed in the wild type and HpHpR KO T.b. brucei. Due to disrupted HpHb import, catalase was active only in the wild type T. b. brucei. Importantly, the absence of HpHbR in pleiomorphic T. b. brucei completely abolished their differentiation in vitro and in vivo, suggesting a key role of HpHbR in the life cycle progression. Moreover, we were able to recapitulate the same phenomenon in the wild type T. b. gambiense who partially produced stumpy forms only after heterologous expression of the T. b. brucei HpHbR. Combined, we demonstrate that in the absence of HpHbR the heme-based metabolism is non-functional in the bloodstream form trypanosomes, causing its failure to differentiate in the mammalian host.

45 Mitochondrial Intermembrane Space Protein Tim54: A crucial component required for the import of Metabolite Carrier Proteins in Trypanosoma brucei.

Ujjal K. Singha, Anuj Tripathi, Aparajita Saha, Joseph T. Smith, Tanusree Singha, and Minu Chaudhuri. Meharry Medical College, Nashville TN 37208. [*Corresponding author]*

The translocase of the mitochondrial inner membrane (TIM) imports a majority of the nucleus-encoded proteins those are destined to the matrix, inner membrane (IM) and the intermembrane space (IMS). Trypanosom brucei, the infectious agent for African trypanosomiasis, possesses a unique TIM complex. We showed that TbTim17, the major component of the T. brucei TIM complex, associates with several novel trypanosome-specific Tims. Here, we characterized the functions of such a novel proteins, TbTim54. TbTim54 didn't show any homology with the fungal Tim54 protein. TbTim54 has been found as a peripherally associated IM protein exposed in the IMS. Blue-Native gel electrophoresis revealed that TbTim54 is present in a protein complex of ~1100 kDa similar to TbTim17. TbTim54 knockdown dissociated the TbTim17 complex and its level was reduced, suggesting TbTim54 is a component of the TbTIM. Import and assembly of the mitochondrial carrier proteins, MCP3, MCP5, and MCP11, was inhibited more due to knockdown of TbTim54 than knockdown of TbTim17, indicating that TbTim54 is primarily involved in the import of internal signal-containing MCPs. TbTim54 is capable to directly interact with two out 6 small Tim proteins, Tim13 and Tim8/13, localized in the IMS and also associated with TbTim17. Together, our data suggest that TbTim54 is a component of TbTim17 complex and is required for the translocation of MCPs into mitochondria. Supported By RO1AI125662

46 Modeling of Trypanosoma brucei population dynamics in mice reveals slow-growing parasites in adipose tissue

Figueiredo, Luisa M Trindade, Sandra; Sequeira, Mariana (Instituto de Medicina Molecular); Dejung, Mario; Bento, Fabio (Institute of Molecular Biology); Pinto-Neves, Daniel (Instituto Gulbenkian de Ciencia); Butter, Falk (Institute of Molecular Biology); Bringaud, Frederic (Universite de Bordeaux); Gjini, Erida (Instituto Gulbenkian de Ciencia)

The adipose tissue is a large reservoir for Trypanosoma brucei in mice. In this environment, adipose tissue forms (ATFs) rewire their gene expression and activate fatty acid catabolism. How a large reservoir of functionally distinct parasites contributes to the dynamics of parasite load in the host remains unknown. We mathematically modeled the number of parasites and the proportion of transmissible forms in blood and adipose tissue during infection. We tested several variables, including parasite growth, differentiation and migration between tissues. The model that best fits our data suggests that in the adipose tissue parasites grow about 38% more slowly than in the blood. To test this model, we compared the proteomes of parasites isolated from the blood and adipose tissue. We identified 2800 protein groups, 6% of which are differentially expressed. Many of the proteins downregulated in ATFs are involved in protein synthesis and ribosome biogenesis. To measure protein synthesis, we performed FACS analysis of a parasites labelled with a methionine analog. We observed that protein synthesis in ATFs is on average 1.8-fold lower than bloodstream forms and 1.7-fold higher than procyclic forms. Moreover, when we compared the cell cycle profile, we observed that in the adipose tissue there is a smaller proportion of parasites in G2/M (16% in fat versus 26% in blood) and a larger proportion of parasites in G1/G0 (76% in fat versus 65% in blood). We conclude that ATFs have a reduced protein synthesis and replicate more slowly than their bloodstream counterparts, thus confirming the predictions of the mathematical model. Slow-growing parasites may help reduce disease severity and contribute to asymptomatic disease described in the field.

47 Molecular and functional characterisation of Leishmania casein kinase 1 provides evidences for a role in endocytosis.

Martel, Daniel Ndiaye, Paya; Pine, Stewart; Späth, Gerald; Rachidi, Najma (Institut Pasteur and INSERM U1201, Unité de Parasitologie moléculaire et Signalisation, Paris)

In mammalian hosts, Leishmania resides in the phagolysosome of macrophages. To ensure its survival, it releases various effectors into the host cell, via extracellular vesicles (EVs). Despite their importance in host-parasite interactions, only few secreted effectors have been characterized and little is known about the mechanisms required for their export into the host cell. Leishmania casein kinase 1.2 (LmCK1.2), a signalling kinase, has been identified in EVs and shown to be important for intracellular parasite survival. To better understand the functions of LmCK1.2 and the mechanisms leading to its export, we used multiple approaches including microscopy, mass spectrometry, and genome editing to characterise its localization and its regulatory domains, to identify its interacting partners, and to generate tagged and knockout parasites, respectively. We showed that LmCK1.2 is ubiquitously distributed in the cytoplasm and associated with the cytoskeleton, for instance at the basal body or at the flagellar pocket. We showed that the C-terminal domain, which contains low complexity regions associated with protein-protein interactions, is essential for these localizations. We identified LmCK1.2 binding partners in both life stages and showed that LmCK1.2 interactions are stage-specific. We selected proteins involved in trafficking as they could contribute to LmCK1.2 export into the host cell. We used CRISPR-Cas9 to either generate null mutants or tagged versions and performed phenotypic and localization studies. Three of these proteins displayed a flagellar pocket localisation and the corresponding null mutants presented an endocytosis defect, which led to growth defect in amastigote. Our findings are consistent with a role of LmCK1.2 in the regulation of endocytosis and thus in protein trafficking.

48 New approaches to studying the GPI biosynthesis pathway in T. brucei: uncovering the missing links.

Ji, Zhe Tinti, Michele; Duncan, Samuel; Guther, Lucia; Ferguson, Micheal (University of Dundee)

The bloodstream form of Trypanosoma brucei (T. brucei) is coated with glycosylphosphatidylinositol (GPI) anchored variant surface glycoprotein (VSG). These GPI anchored VSG homodimers act as the first line of defence for the parasites and undergo antigenic variation leading to persistent infection. The bloodstream form parasite also expresses an essential GPI-anchored transferrin receptor.

A lot is known about GPI anchor biosynthesis in T. brucei, indeed the first studies of GPI anchor structure and biosynthesis were performed on this organism and these methodologies were subsequently applied to mammalian cells, yeast and other organisms. Despite conservation in the core structure of almost all GPI anchors across eukaryotic evolution, notable differences occur between the T. brucei and mammalian GPI anchor biosynthetic pathways making this pathway a possible a drug target.

Here we present quantitative proteomics approach to try to identify proteins that catalyse key steps of GPI anchor biosynthesis in T. brucei but are still unknown ("missing links"). Known components in this pathway were epitope tagged with Myc sequences and co-immunoprecipitation (co-IP) was conducted with and without chemical cross-linking using anti-Myc agarose beads. Associated proteins (putative "missing links") were identified by label-free and SILAC quantitative proteomics, respectively.

In this poster, I present data on the identification of proteins associated with TbdeAc2, the GPI anchor inositol deacylase enzyme (1). In situ C-terminal tagging of TbdeAc2 with a 3Myc sequence allowed its immunoprecipitation with anti-Myc beads. Native-PAGE and Western blotting of the IP showed that TbdeAc2-3Myc was present in a high-molecular weight complex. Subsequent experiments using SILAC quantitative proteomics identified a single predominant protein that co-immunoprecipitated with TbdeAc2-3Myc. The same protein was also identified an experiment using chemical cross-linking (to preserve membrane protein interactions) followed by anti-Myc pull-down and label-free proteomics. This protein may represent a "missing link" in the GPI-biosynthetic pathway, or a regulatory protein of TbdeAc2. We'll perform a reverse pull down of this co-IP protein to confirm its association with TbdeAc2 and perform functional studies to understand its role in the T.brucei GPI-biosynthetic pathway.

1. Hong, Y., Nagamune, K., Morita, Y. S., Nakatani, F., Ashida, H., Maeda, Y., and Kinoshita, T. (2006) Removal or maintenance of inositol-linked acyl chain in glycosylphosphatidylinositol is critical in trypanosome life cycle. J Biol Chem 281, 11595-11602

49 Obtaining a high-quality, phased genome of Trypanosoma brucei and what to do with it

Brink, Benedikt Cosentino, Raul; Müller, Laura; Siegel, Nicolai (LMU Munich)

To study the mechanisms underlying antigenic variation, we choose Trypanosoma brucei, a diploid parasite that contains a vast repertoire of more than 2000 genes coding for antigens. Although Lister 427 is the most widely used isolate, only the genome of the TREU 927 isolate had been fully assembled and served as reference genome. However, previous studies have revealed that the content and extension of the subtelomeric VSG arrays is completely different between these strains, being much larger in the Lister 427 strain.

To obtain a de novo phased T. brucei genome assembly, where both homologous chromosomes are assembled separately, we combined long read sequencing (PacBio) and genome-wide chromosome conformation capture (Hi-C) data. The long reads were assembled into contigs and the Hi-C data was used for scaffolding.

The finished genome yielded a number of surprising discoveries. For example, bloodstream expression sites (BES) and VSG arrays seem to exclude each other at chromosome ends.

Following the assembly of the phased genome, we were able to perform several novel downstream analyses. We calculated the frequency of genomic variants between the two alleles of a chromosome and discovered an intriguing pattern of regions with high variability and regions with very low variability. To our surprise, when mapping RNA-seq data to the phased genome, we discovered that ‘our’ wild type Lister 427 isolate contained an additional copy of chromosome 5. The fact that transcripts from chromosome 5 are homogenously more abundant also provides first evidence against allele specific expression in T. brucei. However, this has yet to be validated with single cell data.

In this study, we demonstrate how to obtain a high quality, phased genome with a combination of long read sequencing and Hi-C. Currently, we are using our pipeline to assembe further T. brucei isolates and try to answer questions about the T. brucei that could not be answered before, e.g. whether allele specific expression is restricted to the VSG expression.

50 Non-invasive monitoring of drug action: Exploring a new assay design for Chagas' disease in vitro drug discovery

Fesser, Anna Braissant, Olivier (Department of Biomedical Engineering, University Basel, Switzerland); Rocchetti, Romina (Swiss Tropical & Public Health Institute, University of Basel, Switzerland); Olmo, Francisco; Kelly, John (London School of Hygiene and Tropical Medicine, London, UK); Mäser, Pascal; Kaiser, Marcel (Swiss Tropical & Public Health Institute, University Basel, Switzerland)

The high number of relapses in posaconazole-treated Chagas' disease patients in the clinical phase II trial published in 2014 posed a number of questions to Chagas' disease drug discovery research: How does Trypanosoma cruzi escape the host's immune response or survive drug pressure? Are there inert, non-replicating - dormant - stages? Is replication deterministically regulated or does it happen stochastically? It became clear that the regulation of replication of T. cruzi amastigotes needs to be studied in more detail.

Currently, we are exploring a new assay design. We are employing a green fluorescent T. cruzi line for live imaging. An innovative plate setup enables us to follow amastigote replication in four hour intervals over a period of 6 or 10 days.

Indeed, we can follow the change in parasite numbers per host cell over time after infection. The results from the live imaging are compared to the results from imaging after fixation and nuclear staining. With these results, we can model the influence of several parameters on in vitro intracellular replication of T. cruzi. Additionally, we can follow parasite numbers per host cell over time of drug exposure. This enables us to determine the time to kill. We can also monitor the decrease of the IC50 value over time of drug exposure.

This innovative assay design provides data to enrich the pharmaco-dynamic profile of drug candidates. Moreover, it builds a basis for a new approach to develop and test new models of the regulation of replication in T. cruzi.

51 Orally delivered gold(I) complexe combined with miltefosine reduces treatment scheme by half on experimental leishmaniasis

Monte-Neto, Rubens Tunes, Luiza (Instituto René Rachou - Fiocruz Minas, Belo Horizonte, Brazil); Garcia, Adriana (Universidade Federal de Juiz de Fora, Juiz de Fora, Brasil); Schmidtz, Vinicius; dos Santos, Helio (Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil); Silva, Heveline; Frézard, Frédéric; Barros, André Luis (Universidade Federal de Minas Gerais, Belo Horizonte, Brazil)

The drugs currently used to treat leishmaniasis have limitations concerning cost, efficacy and safety, making urgent the search for new therapeutic alternatives. On previous in vitro drug screening, using auranofin analogues gold(I) complexes, we have selected two most active compounds to evaluate in vivo efficacy. Here we shown that gold(I) complexes AdT Et (triethylphosphine[(methyl-1-adamantane)1,3-thiazolidine-2-thione(κS)]gold(I)) and AdO Et (triethyllphosphine[5-adamantyl-1,3,4-oxadiazole-2-thiolate(κS)]gold(I)) act by inhibiting trypanothione reductase enzyme and exerts in vivo efficacy. BALB/c mice infected with luciferase-expressing L. braziliensis or L. amazonensis and treated orally with 12.5 mg/kg/day of AdT Et or AdO Et, during 27 days, presented reduced lesion size and parasite burden, as revealed by bioimaging. Combination of AdT Et and miltefosine allowed for a 50 % reduction in miltefosine treatment time. The maximum Au serum concentration was 2.5 and 3.4 mg/L, with Tmax of 8.8 and 14.6 h, and Au elimination half-life of 26.4 and 31.6 respectively for AdT Et and AdO Et. No alteration in ALT, AST, urea or creatinine was observed in treated mice. Thus, complexes AdT Et and AdO Et presented favorable pharmacokinetic and toxicity profiles that encourage further drug development studies. Gold(I) complexes are promising antileishmanial agents, with potential for therapeutic use, including in leishmaniasis caused by antimony-resistant parasites. Financial Support: CNPq, CAPES, Fapemig.

52 Population genomic analysis of Leishmania infantum in Piauí, Brazil

Forrester, Sarah Carnielli, Juliana (York Biomedical Research institute, Department of Biology, University of York, United Kingdom); Costa Silva, Vladimir (Laboratório de Pesquisas em Leishmanioses, Instituto de Doenças Tropicais Natan Portella, Universidade Federal do Piauí, Teresina-PI, Brazil); James, Sally (1. Technology facility, Genomics facility, University of York, United Kingdom); Costa, Dorcas; Costa, Carlos (Laboratório de Pesquisas em Leishmanioses, Instituto de Doenças Tropicais Natan Portella, Universidade Federal do Piauí, Teresina-PI, Brazil); Mottram, Jeremy; Jeffares, Daniel (1. York Biomedical Research institute, Department of Biology, University of York, United Kingdom)

Leishmania infantum is responsible for the most severe form of leishmaniasis, visceral leishmaniasis (VL), in Brazil. Piauí (PI) state is a focus of intense transmission of L. infantum in Brazil. Our previous study identified parasite genotype differences that correlate with patient outcome (cure or relapse), during a clinical trial for an oral based drug, miltefosine (Carnielli et al., 2018). Previous population genetic studies from microsatellite data have shown complex population structures within Brazil at both a state, and country wide level (Kuhls et al., 2013; Motoie et al., 2013). In order to understand the parasite genomic component of patient treatment outcome, and more fully understand the genomic landscape of Brazil, we have embarked on the first large L. infantum population study using genomic sequencing. Analysis of 133 sequenced samples from Piauí allow us to describe the diversity within this region. We have identified strong signatures of genes that are undergoing balancing selection, a hallmark of host-parasite interactions. By comparative analysis of L. infantum strains from Piauí and L. donovani strains from India and Ethiopia; we will understand the mechanisms driving parasite evolution in Leishmania. We have also shed light onto the degree of diversity and expansion in Brazilian L. infantum through the identification of population structure at a high resolution for the first time using whole genome sequencing data.

POSTERS: Session B
Monday 7:00 pm

04/29/19

53 Proteomics in kinetoplast species

Butter, Falk

Mass spectrometry-based proteomics is a powerful tool to study the expression of thousands of proteins in a single experiment. Especially for trypanosomes, we have shown that the transcriptome is only a moderate proxy of the proteome, likely due to their polycistronic expression units.

We have used it in the last years to help studying different biological questions in trypanosomes: the existence of small proteins (Ericson et al., BMC Biol 2014), differences of the proteome in different stages and during developmental differentiation of the bloodstream to the procyclic form in vitro (Butter et al., MCP 2013; Dejung et al., PLOS Path 2016), and determination of nuclear proteins and proteins of the nuclear periphery granules by comparative enrichment (Goos et al., PLoS One 2017; Goos et al., NAR 2019).

Recent yet unpublished applications will be presented: proteomics of parasites undergoing in vitro induced differentiation from procyclic to metacylic trypanosomes comparing transcriptome and proteome during the process (with Alena Zikova, Budweis), measurement of the proteome for restricted sample material on the example of trypanosomes isolated from mouse tissues (with Luisa Figueiredo, Lisboa) and streamlined analysis of the VSG coat composition (with Christian Janzen, Wuerzburg). While also extension to other kinetoplast species like Leishmania will be shown, we would like to engage with the community for further opportunities.

54 RNA quality control in mitochondria of Trypanosoma brucei

Afasizheva, Inna

Most mitochondrial mRNAs in Trypanosoma brucei undergo massive U-insertion/deletion editing to create open reading frames. These sequence changes disrupt collinearity between the genome and transcriptome and introduce considerable heterogeneity into mRNA pool. Here, we report recent advances in understanding the polyadenylation-based surveillance mechanisms that ensure translation of correctly edited mRNAs. Addition of short 3′ A-tail by mitochondrial KPAP1 poly(A) polymerase prior to editing protects mRNA from 3′-5′ degradation during the editing process. Conversely, completion of editing is manifested by A-tail extension into long A/U-heteropolymer. This unconventional mRNA modification stimulates translation by increasing mRNA affinity to the small ribosomal subunit. The distinct roles and editing-dependent temporal separation of A-tailing and A/U-tailing events imply existence of sequence-specific factors that sense the mRNA's editing status and regulate 3′ additions. We identified pentatricopeptide-repeat containing (PPR) RNA binding proteins responsible for monitoring mRNA editing status, 3′ modifications, and direct binding to the ribosome. We show that Kinetoplast Polyadenylation Factor 3 (KPAF3) specifically recognizes 3′ end of pre-edited transcripts thereby stabilizing mRNAs, and stimulates polyadenylation. Initiation of editing displaces KPAF3 leaving mRNA reliant on short A-tail as stability determinant. We further show that Kinetoplast Polyadenylation Factor 4 (KPAF4) recognizes a stretch of five adenosines acting as poly (A) binding protein. In this capacity, KPAF4 blocks 3′-5′ mRNA degradation of adenylated mRNAs by the mitochondrial processome and limits their premature uridylation by RET1 TUTase. The latter prevents translational activation of partially-edited mRNAs. Furthermore, we demonstrate that upon completion of editing KPAF1/2 heterodimer recruits KPAP1 and RET1 thereby inducing long A/U-tail addition specifically to fully-edited mRNA. Finally, we identify PPR factors that represent integral ribosomal proteins involved in direct recognition of specific mRNAs. Collectively, our findings reveal previously unappreciated roles of PPR proteins as polyadenylation factors, and poly(A) binding, and ribosomal proteins.

55 RNA viruses in trypanosomatids

Yurchenko, Vyacheslav Grybchuk, Danyil; Kostygov, Alexei; Macedo, Diego (Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic); Lukeš, Julius (Institute of Parasitology, Biology Centre, and Faculty of Science, University of South Bohemia, C?eské Bude?jovice (Budweis), Czech Republic)

Knowledge of viral diversity is expanding greatly, but many lineages remain underexplored. In this work we analyzed presence of RNA viruses in kinetoplastids. First, we surveyed viral presence in about 70 cultured monoxenous trypanosomatids. Leptomonas pyrrhocoris was a hotbed for viral discovery, carrying a virus (Leptomonas pyrrhocoris ostravirus 1) with a highly divergent RNA-dependent RNA polymerase missed by conventional BLAST searches, an emergent clade of tombus-like viruses, and an example of viral endogenization. A deep-branching trypanosomatid viral lineage showing strong affinities to bunyaviruses was termed "Leishbunyavirus" (LBV) and judged sufficiently distinct to warrant assignment within a proposed family termed "Leishbunyaviridae". A divergent LBV was also found infecting dixenous Leishmania (Mundinia) martiniquensis. Despite extensive sampling, we found no relatives of the totivirus Leishmaniavirus in Leishmania relatives (members of the genera Crithidia and Leptomonas), but, unexpectedly, documented their presence in flea-infecting trypanosomatids of the genus Blechomonas. Numerous relatives of trypanosomatid viruses were found in insect metatranscriptomic surveys, which likely arise from trypanosomatid microbiota.

Our data shed important insights on the emergence and evolution of trypanosomatids viruses. As they were documented in over a quarter of isolates tested, many more are likely to be found in the over 600 unsurveyed trypanosomatid species. Additional work is needed to estimate the extent of viral diversity and the role viruses may be playing in trypanosomatid biology.

This work was supported by the European Regional Development Funds (16_019/0000759).

56 Genome-wide screening for modifiers of an anaphase entry mutant provides insights into the trypanosome kinetochore regulatory network

Brusini, Lorenzo Wickstead, Bill (University of Nottingham)

Faithful genome segregation during mitosis requires regulated attachment between the spindle and newly duplicated chromosomes. Kinetochores form this attachment and reversible phosphorylation of specific components ensures correct chromosome movement. In model systems a phosphorylation gradient involving Aurora and Mps1 kinases and PP1/PP2A phosphatases signals the Spindle Assembly Checkpoint (SAC), a surveillance mechanism that prevents anaphase entry until kinetochores are bi-orientated.

Mitosis in trypanosomes differs from canonical models in several ways. Despite the need to segregate ~120 chromosomes, fewer than 10 kinetochore-like structures are visible during mitosis in Trypanosoma brucei. In addition, trypanosome kinetochores are highly divergent in sequence and composition from models. As with most kinetochore proteins, SAC components have not been readily identifiable in trypanosomes and it has been suggested this checkpoint itself may be absent.

We recently identified KKIP7 as a phosphatase that accumulates specifically at metaphase kinetochores. Here we show that KKIP7 is a member of a family of phosphatases not present in models, but found in several other eukaryotic lineages, and closely related to PP1. Disrupting native KKIP7 function by displacement with a phosphatase-dead form lengthens mitosis by specifically delaying entry into anaphase. To investigate this further, we created a toolkit to allow tunable, inducible expression of mutant transgenes in combination with RNAi and performed a genome-wide screen (240,000 mutants) for suppressors/enhancers of mutant kkip7 in the first high-throughput screen for epistasis in trypanosomes. This demonstrated epistatic interactions with spindle motors, kinetochore proteins (including the Ndc80-like protein, KKIP1) and Aurora complex components – all highly suggestive of a “hidden” signalling network in trypanosomes working in a manner very similar to model systems.

57 Role of the telomere binding protein TbUMSBP2 in chromatin remodeling in trypanosomes

Soni, Awakash Klebanov-Akopyan, Olga; Shlomai, Joseph (Hebrew University, Jerusalem, Israel)

DNA replication, maintenance and gene expression require the decondensation or “opening” of the condensed DNA to enhance its accessibility to the replication and transcription machineries. Universal Minicircle Sequence Binding Protein 2 (UMSBP2) is a CCHC-type zinc-finger protein that has been previously found to function in the replication and segregation the mitochondrial (kinetoplast) DNA (kDNA) and to be essential for telomeres stability and integrity. In a previous study we have found that the interactions of mitochondrial UMSBP with kinetoplast-associated histone H1-like proteins (KAPs) resulted in the decondensation of the kDNA network. Here, we have explored the possibility that the nuclear T. brucei UMSBP (TbUMSBP2) may interact with T. brucei nuclear histones and studied the potential consequences of these interactions. Yeast two hybrid analysis, as well as protein-affinity chromatography revealed specific protein-protein interactions between TbUMSBP2 and core histones H2A, H2B and H4. Fluorescence microscopy and biochemical analyses demonstrated that these protein-protein interactions resulted in the decondensation of DNA condensed by core histones and by histone H1. Furthermore, challenging TbUMSBP2 capacity to remodel the complete genome of the metazoan eukaryote Xenopus laevis demembraned sperm chromatin, has led to remodeling of its packed chromatin. It is suggested that TbUMSBP2 has the potential capacity to function in vivo as a chromatin remodeler that renders it accessible to the replication and transcription machineries. These observations demonstrate the remodeling of a condensed kDNA/chromatin via specific interactions of histones with the replication initiator and telomere binding protein TbUMSBP2, a route for genome remodeling, which may be common to both the mitochondrial and nuclear genomes in trypanosomes.

58 Stage-specific function of Alba3 in Trypanosoma brucei

Bevkal Subramanyaswamy, Shubha Heller, Manfred (Head of Proteomics & Mass Spectrometry Core Facility, University of Bern); Naguleswaran, Arunasalam; Roditi, Isabel (Institute of Cell Biology, University of Bern)

Alba-domain proteins have a variety of functions ranging from DNA-binding in Archaea to tRNA processing in mammalian cells and translational control in protozoan parasites.T. brucei has four Alba-domain proteins that have been shown to interact with each other and with proteins of the translation machinery1. Alba3 appears to have a pivotal role, since knocking it down by RNAi leads to depletion of Albas1&2 in procyclic forms. Although RNAi is efficient, only mild growth defects are observed. By performing a knockout of Alba3 (Alba3KO) in pleomorphic bloodstream forms, we demonstrated that Alba3 is not essential for growth of long slender forms and that the knockout can differentiate to the stumpy form. When stumpy forms are triggered to differentiate to procyclic forms, Alba3KO expresses GPEET and EP procyclins with the same kinetics as the wild type, but subsequently undergoes growth arrest. To assess this phenotype, we conducted comparative proteomic analysis of Alba3KO and wild-type parasites. Interestingly, we see a large number of proteins that are differentially expressed in wild-type and Alba3KO during differentiation. Several proteins specific for bloodstream forms persist in Alba3KO, while many mitochondrial proteins are depleted or undetectable. Interestingly, the RNA binding protein RBP6, which is normally expressed in proventricular forms, is detectable in the knockout 6 days after triggering differentiation. Transcriptomic analyses of the wild-type and Alba3KO during differentiation are currently being performed. Taken together, we hypothesize that Alba3 plays large scale role in the regulation of proteins that are relevant to specific stages of parasite development.

1Mani et al., PLoS One 2011.

59 Structural and Mechanistic insight into Trypanosoma brucei Telomerase RNA

Dey, Abhishek Saha, Arpita; Li, Bibo (Cleveland State University); Chakrabarti, Kausik (University of North Carolina at Charlotte)

The telomerase enzyme counterbalances the progressive loss of DNA at the chromosome termini (also known as 'telomere') by adding DNA repeats onto chromosome ends. This telomeric repeat addition property of telomerase stems from the reiterative utilization of the short internal 'templating' sequence of the integral RNA component, which is an extraordinary feature for an RNA dependent DNA polymerase. This processive utilization of RNA template by telomerase reverse transcriptase follows a specialized mechanism. Indeed, species-specific RNA-protein interactions in the telomerase ribonucleoprotein results in unique telomeric repeats in vertebrates, yeast and plants. However, the mechanistically varied processes of telomere lengthening which requires active telomerase is known only for handful of organisms. This situation is more complex and intriguing in certain instances. For example, Trypanosomes make identical telomeric repeat sequences like humans using a vastly different 'template' domain in their RNA molecule. To understand this unique molecular mechanism involved in telomere repeat synthesis in T. brucei, we sought to determine the structure of telomerase RNA catalytic core domain in wild type and genetic mutants of T. brucei using Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) assay for both in vivo and in vitro RNAs. Our results show that the catalytic core can adopt strikingly different structures in the presence and absence of its cognate protein. In addition, unique tertiary interactions between different domains of telomerase RNA occurs which may allow the timely accessibility of RNA to and utilization of telomeric DNA substrates. Altogether, our results will provide initial imprints of T. brucei telomerase RNA structure dynamics which entails important processive steps in telomere length homeostasis in this clinically relevant kinetoplastid species.

60 Structure of T. brucei CC2D suggests an auto-regulatory membrane binding of its C2 domain

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

Background: Trypanosoma brucei is the causative agent of sleeping sickness in Africa. TbCC2D is a protein essential for cell proliferation and is localized to both the flagellum attachment zone (FAZ) and the FAZ-associated ER in T. brucei. The protein contains multiple predicted coiled-coil motifs at its N-terminus and a putative C2 domain with an enormously long extension (40 residues) at its C-terminus. We set out to find out the structure of the C2 domain and the function of the long extension at its C-terminus.

Results: We have determined a 1.7-Å resolution crystal structure of the C-terminal C2-containing region of TbCC2D (aa709-906). The structure reveals that the C2 domain dose not bind any calcium ions, but rather contains an unusual cluster of three conserved basic patches on one side of its surface. The basic cluster is covered by a negatively charged hook-like structure, which is formed by the C-terminal 40-residue extension. Another crystal structure of the C2 domain alone (aa709-865, 1.5-Å resolution) shows the same fold as the C2 domain together with the “hook”, suggesting that the C2 domain is structurally independent of the “hook”. Mutations of the conserved acidic residues on the “hook” disrupt its interaction with the C2 domain. We predict that TbCC2D might oscillate between a “closed” and an “open” conformation to respectively hide and expose the positively charged patches, which would allow TbCC2D to selectively target to its destination membrane to control its function in the cell. The hypothetical working model, which is currently being validated, would provide an unprecedented auto-regulatory mechanism of membrane binding of a C2 domain-containing protein.

61 Systematic Analysis of potential histone mark writers, readers and erasers in Trypanosoma brucei

Carloni, Roberta Staneva, Desislava; Tong, Pin; Auchynnikava, Tatsiana; Matthews, Keith; Allshire, Robin (University of Edinburgh)

The basic units of chromatin are the nucleosomes, formed by DNA and core histones. Histone tails are subjected to post-translational modifications (PTMs) associated with transcriptionally active euchromatin or repressive heterochromatin. In eukaryotes, formation of constitutive heterochromatin is usually mediated through H3K9 methylation by SET-domain proteins, which is read by chromo-domain proteins. Electron microscopy analyses have shown that as in other eukaryotes heterochromatin clusters around the periphery of trypanosome nuclei. Since T. brucei histones are divergent from other eukaryotes and lack a lysine residue equivalent to eukaryotic H3K9, heterochromatin must be formed by a distinct mechanism in this evolutionarily divergent parasite.

To obtain insight into the mechanism of heterochromatin formation in African trypanosomes we performed systematic YFP in-situ tagging of most identifiable putative writers, readers and erasers of histone modifications in bloodstream form parasites, comprising approximately 70 proteins. ChIP-seq (Chromatin immunoprecipitation and sequencing) analyses showed enrichment of a subset of nuclear bromodomain and HAT proteins near transcription start sites (TSS). Our results confirm and extend published analysis demonstrating that these proteins accumulate at H4K10Ac marked TSSs, and regions that exhibit open chromatin. Detailed Co-IP and LC-MS/MS proteomic analyses of a subset reveal interactions between Bromo3, Bromo5 and HAT2; Bromo1 and Bromo4; Bromo6, HAT1 and NuA4; NuA4 and PHD1. In contrast, of the 30 identifiable T. brucei SET-domain proteins with unknown function, only 7 exhibited some nuclear localisation. Proteomics on 5 of them detected no interactions with other readers, writers or erasers. Understanding the combinatorial interactions of these potential histone modifiers should determine their role in defining distinct chromatin structures and functions in T. brucei and how these differ from conventional eukaryotes.

62 T. brucei RAP1 has a DNA binding activity that is important for VSG monoallelic expression.

Afrin, Marjia Gaurav, Amit (Cleveland State University); Yang, Xian (The Hong Kong Polytechnic University); Sandhu, Ranjodh (Cleveland State University); Zhao, Yanxiang (The Hong Kong Polytechnic University); Li, Bibo (Cleveland State University)

Trypanosoma brucei causes human African trypanosomiasis and regularly switches its major surface antigen, VSG, to survive the host immune response. VSGs are monoallelically expressed from subtelomeric expression sites. We have shown that telomere proteins affect VSG silencing and switching. Specifically, TbRAP1 associates with the telomere chromatin, is essential for VSG silencing, and suppresses DNA recombination-mediated VSG switching. Additional observations from our lab further indicate that localization of TbRAP1 at the telomere is critical for VSG regulation. However, how TbRAP1 is recruited to the telomere is unknown. Among all known RAP1 homologues, only budding yeast RAP1 exhibits a strong duplex telomere DNA binding activity that is mediated by its central Myb and Myb-like domains, because usually two Myb domains are required for DNA recognition. Human RAP1 has a single Myb domain and does not seem to bind telomere DNA directly in vivo. TbRAP1 has a similar domain structure as human RAP1 and contains only one central Myb domain, which is unlikely to bind DNA based on sequence analysis. Surprisingly, we found that TbRAP1 has a DNA binding activity in vitro that is independent of its Myb domain. Additionally, localization of TbRAP1 to the telomere is independent ofTbTRF, which binds the duplex telomere DNA and interacts with TbRAP1, suggesting that TbRAP1's DNA binding activity is sufficient to localize it to the telomere. Importantly, we found that TbRAP1 mutants that abolish the DNA binding activity lead to acute cell growth arrest and severe VSG derepression, indicating that this DNA binding activity is essential for TbRAP1's function in antigenic variation and cell viability.

63 The ‘essential genome’ differs substantially between monomorphic and pleomorphic trypanosomes

D'Archivio, Simon Whipple, Sarah (University of Nottingham); Trindade, Sandra; Figueiredo, Luisa (Instituto de Medicina Molecular); Wickstead, Bill; Gadelha, Catarina (University of Nottingham)

As the primary interface between parasite and host, the cell surface of Trypanosoma brucei is of outstanding biological and therapeutic importance. We previously defined and validated a high-confidence a cell surface proteome (surfeome) for bloodstream-form T. brucei. To intersect these localisation data with phenotypic information, it is necessary to be able to define the ‘essential surfeome’, both in culture and in animals, and understand differences between lab models. Mining the RNA interference target-sequencing (RIT-Seq) dataset for the essential surfeome proven to be of limited predictive power. The majority of surfeome genes (92 out of 175) are missing from the mapped gene set; the ones included and also predicted to create a significant loss of fitness by RIT-Seq did not produce growth defects when validated by individual RNAi.

To study surfeome essentiality we modified a genome-scale approach (DRiF-Seq) to create 1000s of independent RNAi fragments targeting surfeome genes, and generated a recipient line in disease-relevant, pleomorphic EATRO1125 cells. This line (AnTat-TTS) is stable to freeze-thaw, generates stumpy forms and cyclic waves of parasitaemia, and inducibly produces 5,000-10,000 independent clones per transfection. A 24,000-clone surfeome-specific library allowed relative fitness quantification at very high accuracy both in vitro and in vivo, demonstrating that 1/4 surfeome genes are required for normal growth in pleomorphic cells, but also differential effects in Lister 427. To investigate this further, we used AnTat-TTS to generate a 250,000-clone library in a genome-wide assessment of loss-of-fitness in pleomorphic cells. Different selection regimes revealed gene sets involved in adaptation to mammalian hosts and in parasite differentiation, but also unexpected substantial differential essentiality in core cellular processes between pleomorphic and monomorphic trypanosomes.

64 The hook complex-associated protein BOH1 in Trypanosoma brucei cooperates with Polo-like kinase to regulate flagellum inheritance and cytokinesis initiation

Li, Ziyin Pham, Kieu; Zhou, Qing; Kurasawa, Yasuhiro (University of Texas Medical School at Houston)

Trypanosoma brucei possesses a motile flagellum that also determines cell morphology, defines the cell division plane, and mediates cell-cell communications. Inheritance of the newly assembled flagellum during the cell cycle is controlled by the Polo-like kinase homolog TbPLK, which also regulates cytokinesis initiation. TbPLK localizes to multiple cytoskeletal structures, such as the flagellar basal body, the hook complex and the distal tip of the newly assembled flagellum attachment zone (FAZ) filament, and is a key player in the cytokinesis signaling pathway that includes the Aurora B kinase and a cohort of trypanosome-specific cytokinesis regulators, such as CIF1, CIF2 and CIF3. While TbPLK functions at the very upstream of the signaling pathway and recruits numerous downstream cytokinesis regulators, mechanistically how TbPLK is targeted to its subcellular locations to execute its multiple functions remains poorly understood. Here we report a trypanosome-specific protein named BOH1 which interacts with and cooperates with TbPLK to regulate flagellum inheritance and cytokinesis initiation. BOH1 interacts with TbPLK at the hook complex and localizes to an unusual sub-domain in the hook complex, bridging the hook complex, the centrin arm and the flagellum attachment zone filament. Depletion of BOH1 disrupts hook complex morphology, inhibits centrin arm elongation, and abolishes FAZ assembly, leading to flagellum mis-positioning and detachment. Further, BOH1 deficiency impairs the localization of TbPLK and its downstream cytokinesis regulator CIF1 to the cytokinesis initiation site, providing the molecular mechanism for its role in cytokinesis initiation. These findings reveal the roles of BOH1 in maintaining hook complex morphology and regulating flagellum inheritance, and establish BOH1 as an upstream regulator of the TbPLK-mediated cytokinesis signaling pathway.

65 The impact of novel telomere-associated protein complexes on VSG expression site regulation in Trypanosoma brucei

Weisert, Nadine Luko, Katarina; Dejung, Mario; Butter, Falk (Institute of Molecular Biology (IMB), Mainz, Germany); Janzen, Christian J. (Department of Cell and Developmental Biology, Würzburg, Germany)

The survival of Trypanosoma brucei in the mammalian host is based on antigenic variation of the variant surface glycoprotein (VSG) coat. T. brucei encodes a large library of VSG genes, but only one is ever expressed at a time. This single VSG gene is transcribed from one of 15 expression sites (ES). The ES are all present in subtelomeric regions of chromosomes, suggesting that telomere-associated proteins may contribute to transcriptional regulation of VSG expression. Previously characterised telomeric proteins such as TbTRF, TbTIF2, and TbRAP1 have been shown to play a role in subtelomeric VSG gene regulation, but the identification of the complete composition of a telomere protein complex has not yet been achieved. To learn more about the role of telomeres in VSG expression, the discovery of novel telomeric proteins is essential.

Two biochemical approaches were used to identify novel telomeric proteins. First, affinity chromatography using telomeric repeat oligonucleotides was used to pull down telomere-binding proteins from trypanosome lysates. Second, a co-immunoprecipitation with the known telomere protein TbTRF was used to identify interaction partners. 24 potential telomere-associated proteins were significantly enriched using these approaches. The role of a selection of these proteins in antigenic variation and the possibility of stage-specific telomere complex composition will be discussed.

66 The kDNA replication apparatus: novel components and ancestry from mobile elements

KRISHNAN, ARUNKUMAR Burroughs,, A. Maxwell; M. Iyer, Lakshminarayan; L., Aravind (NIH/NLM/NCBI)

Protein 'weaponry' deployed in biological conflicts between selfish elements and their hosts are increasingly recognized as being re-purposed for diverse molecular adaptations in the evolution of several uniquely eukaryotic systems. The anti-restriction protein ArdC, transmitted along with the DNA during invasion, is one such factor deployed by plasmids and conjugative transposons against their bacterial hosts. Using sensitive computational methods, we unify the N-terminal single-stranded DNA-binding domain of ArdC (ArdC-N) to the DNA-binding domains of the Trypanosoma Tc-38 (p38) the principal minicircle origin recognition protein involved in kinetoplast(k) DNA replication and dynamics. Further we unified these to the so-called 'beta-hairpin domains' in the nucleotide excision repair (NER) XPC/Rad4 protein, thereby allowing us to predict their structure. The acquisition of the ArdC-N domain in the common ancestor of the kinetoplastids gave rise to a rapidly diversifying protein family (the Tc-38-like proteins), with multiple duplications and losses of the ArdC-N domain within the same polypeptide. The members of this diverse protein family are predicted to act like Tc-38 (p38) in kDNA binding and dynamics by forming an extended DNA-binding interface that could span a substantial length of kDNA. The observed sequence diversity in the family potentially reflects diversity in binding-target specificity and parallels the emergence of a diversity of kDNA morphs in kinetoplastids. Further, we show that parallel acquisitions of genes not only from plasmids/conjugative elements but also bacteriophages and eukaryotic viruses gave rise to several key kDNA replication components including the Topoisomerase IA, DNA polymerases IB-Ds, and DNA ligases. In summary, we show that a mobile-element-derived DNA-binding and replication components potentially acquired from a plasmid-present in a bacterial endosymbiont of the ancestral of kinetoplastid, were re-purposed for multiple roles in kDNA replication. These results throw light on one of outstanding questions in kinetoplastid-biology: how the plasmid-like replication and unusual structure of the kDNA so distinct from that of the mitochondria of other eukaryotes might have emerged in the kinetoplastids.

67 The Leishmania infantum Miltefosine Sensitivity Locus

Brambilla Trindade Carnielli, Juliana Forrester, Sarah; Jeffares, Daniel C.; Davey, John (York Biomedical Research Institute, Department of Biology, University of York, UK); Costa, Carlos Henrique Neri (Laboratório de Pesquisas em Leishmanioses, Instituto de Doenças Tropicais Natan Portella, Universidade Federal do Piauí, Brazil); Mottram, Jeremy C. (York Biomedical Research Institute, Department of Biology, University of York, UK)

Miltefosine has been used successfully to treat visceral leishmaniasis (VL) in India, but it was unsuccessful for VL in a clinical trial in Brazil (cure rate=60%). We used a genome-wide association study to identify a molecular marker that predicts VL treatment failure following whole genome sequencing of 26 Leishmania infantum isolates, from cured and relapsed patients. The Miltefosine Sensitivity Locus (MSL) in L. infantum has a frequency that varies in a cline from 95% in North East Brazil to less than 5% in the South East (n=188). The MSL was found in the genomes of all L. infantum and L. donovani sequenced isolates from the Old World (n=671), where miltefosine can have a cure rate higher than 93% (Carnielli et al., 2018 EbioMedicine 36:83-91). We have now generated the first Brazilian L. infantum 36 one-contig chromosome reference genome (MHOM/BR/06/MA01A) using nanopore and Illumina sequencing. We show that the MSL contains four genes: 3'-nucleotidase/nucleases LinJ.31.2370 and LinJ.31.2380; helicase-like protein LinJ.31.2380; and 3,2-trans-enoyl-CoA isomerase LinJ.31.2400. To test if any of the genes contributed to natural resistance to miltefosine, the Brazilian L. infantum MSL+ MA01A reference cell line was engineered to express Cas9 and T7, enabling CRISPR/Cas9-mediated genome editing. The whole MSL and each individual MSL gene was then deleted to create a series of mutants. Deletion of the whole MSL and both nucleotidases significantly reduced miltefosine susceptibility of promastigotes in vitro, however, no difference in susceptibility to miltefosine was observed for the intracellular amastigote stage. Consistent with the GWAS, these data indicate that the molecular mechanisms involved in natural resistance to miltefosine in Brazilian L. infantum are multifactorial.

68 The microtubule-associated protein PAVE1 regulates the dynamics of the subpellicular microtubule array in Trypanosoma brucei

Sinclair-Davis, Amy Sladewski, Thomas; de Graffenried, Christopher (Brown University)

Microtubules are essential cytoskeletal filaments present in all eukaryotic cells. Due to their inherently dynamic nature, they are often crosslinked to create stable structures for long-term force propagation. The subpellicular microtubule array of the protozoan parasite Trypanosoma brucei, the causative agent of African sleeping sickness, is a remarkable example of a highly organized microtubule structure. The array underlies the parasite's plasma membrane and maintains its asymmetric cell shape, which is required for its unique motility and pathogenicity. The microtubules in the array are unusually stable and are crosslinked to each other throughout the cell cycle. Despite its prominence in the cellular architecture of T. brucei, little is known about how the array is duplicated and subsequently partitioned during cell replication. We have recently identified a protein termed PAVE1 that localizes to the inter-microtubule crosslinks present at the curved posterior end of the subpellicular array in trypanosomes. PAVE1 depletion truncates the posterior portion of the array, which inhibits cell division. Pulse-chase experiments show that PAVE1 is added to the array in an ordered fashion and suggests that it plays a vital role in stabilizing the microtubules at the curved cell posterior. These results suggest that the subpellicular array is more dynamic than previously thought and that the microtubule crosslinks, which are present throughout the array, may be uniquely configured in different parts of the cell to perform unique functions. Defining how the array is built, maintained, and segregated in T. brucei has broad implications in understanding the biogenesis of complex microtubule structures throughout biological systems.

69 The Trypanosoma brucei infectious form mitochondrion is capable of ATP generation

Dolezelova, Eva Taleva, Gergana (1 Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic 2 Faculty of Science, University of South Bohemia, Ceske Budejovice,); Panicucci, Brian (Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic); Hierro-Yap, Carolina (1 Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic 2 Faculty of Science, University of South Bohemia, Ceske Budejovice,); Husova, Michaela (Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic); Pineda, Erika; Bringaud, Frederic (University of Bordeaux, Bordeaux, France); Zíková, Alena (1 Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic 2 Faculty of Science, University of South Bohemia, Ceske Budejovice,)

It has been a long-running narrative that the reduced functions of the Trypanosoma brucei bloodstream form (BSF) mitochondrion requires the ADP/ATP carrier (AAC) to import ATP that is consumed by the FoF1-ATPase to generate the essential mitochondrial membrane potential (Δym). This view is now being challenged as we have created AAC null mutants that are viable in vitro and retain their virulence in a mouse model. In situ assays reveal that the mitochondrion of these null mutants is not able to establish a Δym when supplied with ATP, thus excluding the possibility of an additional ATP transporter. Furthermore, the AAC null mutants exhibit a 100-fold higher sensitivity to TMPM, an inhibitor of the enzyme positioned immediately upstream of one of the two possible mitochondrial substrate phosphorylation (SUBPHOS) reactions catalyzed by succinyl-CoA ligase (SCoAS). While the SCoAS null mutant is viable in vitro, its virulence was significantly reduced in the mouse model. In vitro, these null mutants display a 40-fold higher sensitivity to an AAC inhibitor, suggesting a greater reliance on the import of cytosolic ATP in the absence of mitochondrial SUBPHOS. In addition, the SCoAS null mutants perished when grown in media containing glycerol, growth conditions that effectively lower the cellular ATP levels compared to a glucose-rich media. Our results suggest the BSF mitochondrion is capable of producing ATP via various enzymatic pathways depending on the available carbon source, thus enabling the parasite to adapt to the diverse niches it may occupy within the mammalian host.

70 Trafficking itinerary of aquaglyceroporin 2 in T. brucei: new insights into the mode of action of pentamidine

Quintana, Juan Field, Mark (University of Dundee)

In T. brucei,intracellular trafficking of surface proteins is essential for the mode of action of several trypanocidal compounds. One clear example is involvement of several components of the endocytic machinery in susceptibility to suramin and ubiquitin-mediated internalisation of ISG75. However, the role of endocytosis in susceptibility to other trypanocidal compounds remains to be further elucidated. Here we demonstrate that T. bruceiaquaglyceroporin 2 (TbAQP2), a type III transmembrane domain protein, also undergoes ubiquitin-mediated internalisation in the bloodstream form of T. brucei. As previously reported for the type I transmembrane protein ISG75, TbAQP2 is post-translationally modified by ubiquitin. Moreover, TbAQP2 forms high molecular weight complexes (~500 kDa), is a short-lived protein(<3h), and degradation seems to be dependent on lysosomal function. Interestingly, there is an overall impairment to lysosomal acidification when cells were exposed to pentamidine, similar to the phenotype observed following Bafilomycin A1 treatment. Furthermore, a concomitant impairment on lysosomal-dependent protein degradation was also observed in pentamidine-treated cells, indicating that pentamidine is likely to disrupt lysosomal function. We conclude that internalisation of TbAQP2 follows a similar, but also distinct, intracellular trafficking itinerary to that of ISG75, leading to lysosomal degradation. We propose a model whereby TbAQP2 internalisation, mediated by direct ubiquitination as reported for other membrane proteins, leads to an accumulation of pentamidine in the lysosome, impairing its function. Our study shows a potential interplay between different intracellular organelles during pentamidine exposure and, in addition to the effects previously reported for loss of mitochondrial membrane potential, provides further insights into the mode of action of pentamidine.

71 Transcription activity contributes to the firing of non-constitutive origins in Trypanosoma brucei maintaining the robustness of the S phase duration

da Silva, Marcelo Santos Cayres-Silva, Gustavo; Vitarelli, Marcela; Marin, Paula (Laboratório Especial de Ciclo Celular, Center of Toxins, Immune Response and Cell Signaling (CeTICS), Instituto Butantan, São Paulo, Brazil); Hiraiwa, Priscila (Plataforma de citometria de fluxo, Instituto Carlos Chagas, FIOCRUZ, Paraná, Brazil); Araújo, Christiane (Laboratório Especial de Ciclo Celular, Center of Toxins, Immune Response and Cell Signaling (CeTICS), Instituto Butantan, São Paulo, Brazil); Ávila, Andrea (Laboratório de Regulação da Expressão Gênica, Instituto Carlos Chagas, FIOCRUZ, Paraná, Brazil); McCulloch, Richard (The Wellcome Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom); Reis, Marcelo; Elias, Maria Carolina (Laboratório Especial de Ciclo Celular, Center of Toxins, Immune Response and Cell Signaling (CeTICS), Instituto Butantan, São Paulo, Brazil)

The co-synthesis of DNA and RNA potentially generates conflicts between replication and transcription, which can lead to genomic instability. In trypanosomatids, eukaryotic parasites that perform polycistronic transcription, this phenomenon and its consequences have not yet been investigated. Here, we showed that the number of constitutive origins mapped in the Trypanosoma brucei genome is less than the minimum required to complete replication within S phase duration. By the development of a mechanistic model of DNA replication considering replication-transcription conflicts, we demonstrated that the activation of non-constitutive origins is indispensable for replication to be completed within S phase period. Moreover, both computational and biological assays pointed to transcription being responsible for activating non-constitutive origins. Together, our results suggest that transcription action through conflicts with replication contributes to the firing of non-constitutive origins, maintaining the robustness of S phase duration. The usage of this increased pool of origins seems to be of paramount importance for the survival of this parasite that infects million people around the world, since it contributes to the maintenance of the replication of its DNA.

72 TRANSCRIPTIONAL CONTROL OF MITOCHONDRIAL GENE EXPRESSION IN TRYPANOSOMA BRUCEI

Afasizhev, Ruslan

Digenetic hemoflagellate Trypanosoma brucei belongs to Kinetoplastea, a taxonomic class defined by possession of a kinetoplast. This nucleoprotein body contains mitochondrial DNA (kDNA) of two kinds: maxicircles encoding ribosomal RNAs, ribosomal proteins and subunits of respiratory complexes, and minicircles bearing guide RNA genes. Relaxed maxicircles and minicircles are interlinked and packed into a dense disc-shaped network by association with histone-like proteins. Decades of kDNA studies unraveled fascinating phenomena of general biological significance, such as DNA bending and mRNA editing, and revealed exquisite details of genome replication, and RNA processing and translation. However, the mechanisms of transcription remain virtually unexplored. Contrary to the enduring view of polycistronic transcription as a prevailing RNA synthesis mode, we present evidence that individual maxicircle protein-coding genes are independently transcribed into 3′ extended precursors. The transcription-defined 5′ terminus is converted into monophosphorylated state by the pyrophosphohydrolase complex, termed the PPsome. Composed of MERS1 NUDIX enzyme, MERS2 pentatricopeptide repeat RNA binding subunit, and MERS3 polypeptide, the PPsome binds to specific sequences near mRNA 5′ termini. Most guide RNAs lack PPsome recognition sites and remain triphosphorylated. RNA editing substrate binding complex (RESC) stimulates MERS1 hydrolase activity and enables an interaction between the PPsome and the polyadenylation machinery. We provide evidence that both 5′ pyrophosphate removal and 3′ adenylation are essential for mRNA circularization, a molecular basis of mitochondrial mRNA stability. Furthermore, we uncover a mechanism by which antisense RNA-controlled 3′-5′ exonucleolytic trimming defines the mRNA 3′-end prior to adenylation. We conclude that mitochondrial mRNAs and rRNAs are transcribed and processed as insulated units irrespective of their genomic location. Importantly, both 5′ and 3′ ends of mature RNAs are defined by transcription initiation. These findings introduce a concept of mitochondrial gene-specific transcriptional control with broad implications in developmental transitions and pathogenesis.

73 Transfer of surface functionality between trypanosome species

Aroko, Erick Jones, Nicola; Engstler, Markus (Würzburg University)

Animal infective African trypanosomes including Trypanosoma brucei, Trypanosoma congolense and Trypanosoma vivax have evolved to differ in tropism, motility and morphology. They can, however, still infect the same hosts and their surface coats are important virulence factors. Of the three species, only T. brucei has been extensively characterized on the cellular and molecular level. The surface of the bloodstream form of this parasite is covered by a monolayer of mainly one densely packed glycosylphosphatidylinositol (GPI)-anchored protein, the variant surface glycoprotein (VSG). T. brucei VSGs have an elongated N-terminal domain that is important for antigenic variation and a shorter, more secluded C-terminal domain that confers flexibility to the protein, thus supporting the coats functionality. T. congolense and T. vivax also express VSGs on their cell surface, but both appear to lack the structured regions found in the C-terminal domain of T. brucei VSGs. T. vivax VSGs are generally much smaller (42-50 kDa) and have been reported to form a less dense coat. We sought to utilize the tractable T. brucei system as a platform for forward surface engineering for a comparative study of the surface coats of African trypanosome species. Whereas a T. congolense VSG could be readily expressed in T. brucei, a T. vivax VSG could only be expressed in T. brucei following slight modifications. The T. brucei based system we have established gives us a perfect model to answer some fundamental questions for example, what happens to exposed T. brucei invariant surface glycoproteins (ISGs) in the smaller trans-species coats. How do different surface components influence VSG diffusion? Will the expression of T. congolense trans-sialidases provide additional function to the T. brucei VSG coat?

74 TriTrypDB: the functional genomics resource for kinetoplastida

Harb, Omar S. Crouch, Kathryn (University of Glasgow); Roos, David (University of Pennsylvania); Hertz-Fowler, Christiane (University of Liverpool)

TriTrypDB.org is supported by the Wellcome Trust (UK) and the NIH (US) and is a free online resource integrating data from multiple 'omics categories and providing analysis tools that enable the development of testable hypotheses based on available data. Moreover, recent implementation of a user workspace allows users to analyze their own data and integrate results into the database for further interrogation. TriTrypDB belongs to the Eukaryotic Pathogen Database Resources (EuPathDB, http://eupathdb.org) family of databases and has been developed in collaboration with GeneDB (http://genedb.org). Currently TriTrypDB includes genomes of 46 kinetoplastida, a number of which undergo manual curation by integration of information from publications, user comments and high throughput assays. Development of an end user annotation platform based on Apollo is on-going and will enable users to provide both functional and structural changes that will appear as community provided annotation pending curatorial review and full integration into the official genome annotation. Additional supported datatypes include transcript-level information (RNA-Seq, microarray, ESTs), protein expression data, epigenomic data (ChIP-chip, ChIP-seq), population-level and isolate data (SNPs, CNV), and functional information from genome-wide RNAi knock-down and fluorescent tagging (TrypTag) analysis. The results of in-house genomic analysis pipeline are also integrated and provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (EC annotations and GO terms), metabolic pathways and evolutionary relationships based on gene orthology (OrthoMCL).

TriTrypDB offers several perspectives for data mining – record pages which compile all data for genes, pathways, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system for querying pre-analyzed data to find genes or features that share biological characteristics; and a private workspace for analyzing primary data (via a Galaxy interface) and exmining it with public data already integrated into EuPathDB.

75 Trypanosome Lytic Factor mediated Protection against Leishmania sp.

Pant, Jyoti Keceli, Mert (Hunter college); Verdi, Joseph (The Graduate Center); Raper, Jayne (Hunter College)

Trypanosome Lytic Factor (TLF) is an innate immune factor against select kinetoplastids. Here we investigated the interaction between TLF and cutaneous Leishmania sp. TLF reduces parasite burden in mice when an intradermal parasite inoculum is used to mimic the sandfly bite. The response depends on the concentration of circulating TLF and the dose of infecting L. major. Depleting neutrophils in the TLF expressing mice exacerbates the infection suggesting that TLF effectively kills parasites within/in concert with neutrophils. In vitro experiments suggest this killing of Leishmania sp. metacyclic promastigotes occurs due to the direct interaction of TLF and Leishmania within the acidic phagosome of phagocytic cells. In vitro we find that metacyclics are killed by a two-step process designed to either mimic neutrophil uptake or macrophage uptake: TLF is first incubated in acidic media (pH 5.6 mimics the neutrophil/macrophage phagosome), which promotes the association of APOL1, the pore-forming protein of TLF, to the metacyclics. Then parasites are switched to neutral media (mimic the escape from neutrophil phagosomes) or further acidification to pH 4.5 (mimics macrophage parasitophorous vacuoles). Parasite numbers are reduced in neutrophil mimicking condition. However, macrophage mimicking condition lead to complete lysis of the parasites. Metacyclics incubated with TLF in neutral pH (mimics extracellular environment) interacts with the parasites but does not lyse them. These results suggest that TLF interaction with metacyclic promastigotes initiates once the parasites are injected intradermally before parasites invade immune cells. Once in immune cells in the acidic phagosome TLF gets activated and kills the metacyclic promastigotes. Amastigotes are resistant to TLF lysis possibly due to downregulated surface glycoproteins.

76 Basalin, a conserved yet evolutionarily unconstrained flagellum protein. Implications for analysis of the Kinetoplastid genomes

Dean, Samuel Moreira-Leite, Flavia; Gull, Keith (University of Oxford)

Most motile flagella have an axoneme that contains nine outer microtubule doublets and a central pair (CP) of microtubules. The CP coordinates the flagellar beat and defects in CP projections are associated with motility defects and human disease. The CP nucleate near a 'basal plate' at the distal end of the transition zone (TZ). Here, we show that the trypanosome TZ protein 'basalin' is essential for building the basal plate and its loss is associated with flagellum paralysis caused by CP nucleation defects and inefficient recruitment of CP assembly factors to the TZ. Sequence analysis did not reveal any non-Trypanosoma basalin orthologs; given the conservation of the basal plate and CP in kinetoplastids and other flagellated eukaryotes this was surprising. However, guided by synteny alone, we identified a basalin ortholog in the related Leishmania species that has conserved synteny, cellular localisation and function but negligible sequence homology. This raises the general concept that proteins involved in cytoskeletal functions and appearing organism-specific, may have highly divergent and cryptic orthologs in other species. Moreover, it reinforces the need for insightful analysis of kinetoplastid genomes.

77 Ubiquitin in the trypanosomes: the Cullin-RING complexes

Canavate del Pino, Ricardo Ono, Kayo (University of Dundee); Zoltner, Martin (Charles University of Prague); Field, Mark C. (University of Dundee)

Despite the growing evidence linking ubiquitination to processes such as immune evasion, host-pathogen interaction and the mode of actions of drugs, these pathways remain largely unexplored among the Kinetoplastids. Previous studies have been able to identify genes that suggest a conserved system between trypanosomes and animals but it was unclear the extent of the conservation and if there is evidence for lineage-specific features. We have used a combined bioinformatic and immunisolation/proteomic strategy to characterise the largest E3 family, the cullin-RING in African trypanosomes. We have identified components of the TbCUL1, the TbCUL4, the TbCUL3 and two apparent trypanosome specific CUL complexes. Proteomic analysis reveals multiple protein abundance changes upon cullin silencing, including defects to the cell cycle. Our data provide a first systematic assessment of cullin composition and functions in the Trypanosoma sp.

78 Unravelling the mode of action of two organometallic anti-T. cruzi compounds

Mosquillo, Florencia Smircich, Pablo (Facultad de Ciencias, Universidad de la República); Gambino, Dinorah (Facultad de Química, Universidad de la República); Garat, Beatriz; Pérez-Díaz, Leticia (Facultad de Ciencias, Universidad de la República)

Our group has been working on the design of new organometallic compounds based on Palladium or Platinum. In particular, two of them have been selected for a deeper study in terms of their mode of action since they have shown low IC50 values as well as very good selectivity indexes against CL Brener T. cruzi epimastigotes. These compounds also affected the infection process as well as the intracellular replication of these parasites since the number of infected cells as well as the number of amastigotes per cell decrease after 24 hours of incubation. The aim of our work is to unravel the mechanism of action of these compounds. In this context, we analyzed their biological effect. Our results suggest that compounds display a trypanocidal mode of action inducing necrosis. Besides, we found a preferential association of the compounds with DNA. To identify genes and/or metabolic pathways affected by the incubation with these compounds and eventually to find molecular targets of them, 'omic' approaches were implemented to study the transcriptomic and proteomic changes promoted after drug treatment. This data allowed to identify potential targets such as NADH-dependent fumarate reductase for the treatment with Pt. To confirm our data, the enzymatic activity of this enzyme was evaluated in presence of Pt compound and a decrease in a dose-dependent manner was observed. Currently, we are working on the validation of this putative molecular target among others through a functional genomic approach and WT parasites were transfected and are being selected to overexpress the selected candidates to validate them as molecular targets of these promising compounds.

79 Widespread roles for the ATR kinase in maintenance of the nucleus and genome of Trypanosoma brucei

Black, Jennifer Ann Crouch, Kathryn; Lemgruber, Leandro; Briggs, Emma; Lapsley, Craig (University of Glasgow); Mottram, Jeremy C (University of York); Tosi, Luiz R O (Universidade de São Paulo); McCulloch, Richard (University of Glasgow)

Genomic damage is a potentially catastrophic event for any cell, since failure to resolve genotoxic lesions can result in malignant transformation or death. As such, cells have evolved myriad pathways by which lesions can be recognized and appropriate repair pathways initiated. At the heart of the damage response resides a family of atypical protein kinases (PKs), of which ATR and ATM have been linked to genome repair in kinetoplastid parasites. However, the full extent of either PK's roles in genome lesion signaling and repair remain understudied. Here we show that RNAi-mediated depletion of ATR in bloodstream form Trypanosoma brucei leads to death, very pronounced accumulation of γH2A (a marker of genotoxic lesions), chromosomal abnormalities and perturbed nuclear structure. To understand this range of effects, we performed immunoprecipitation of N-terminally tagged ATR to identify potential interaction partners: in the absence of genotoxic stress components of the chromosomal segregation machinery were recovered, while in the presence of genotoxic stress nucleoporins and kinesins were additionally identified. Testing these interactions should reveal how ATR acts in chromosome and nuclear structure maintenance. We also performed ChIP-seq of γH2A before and after ATR RNAi and find that loss of the PK causes modified histone accumulation most notably at centromeres and in intergenic regions across all multigenic transcription units. These data are consistent with ATR acting on R-loops, which we have shown form at these loci, and potentially indicate an active role for ATR in the functions provided by these RNA-DNA hybrids. Finally, we will describe the widespread effects of ATR loss on mRNA abundance revealed by differential RNA-seq of RNAi induced and uninduced cells.

80 A CRISPR/Cas9/riboswitch-based method for downregulation of gene expression in Trypanosoma cruzi

Lander, Noelia M. Cruz-Bustos, Teresa; Docampo, Roberto (University of Georgia)

Few genetic tools were available to work with Trypanosoma cruzi until our recent introduction of the CRISPR/Cas9 technique for gene knockout, gene complementation, and endogenous gene tagging. However, methods for the study of essential genes, multigene families or genome-wide functional screenings are still missing, and this has been an obstacle to identify alternative targets for antiparasitic interventions in T. cruzi. The RNA interference pathway, widely used for gene silencing in the related kinetoplastid Trypanosoma brucei, is absent in T. cruzi and attempts to generate conditional gene knockouts in this parasite have been also unsuccessful. Riboswitches are naturally occurring self-cleaving RNAs (ribozymes) that can be ligand-activated. Results from our lab recently demonstrated the usefulness of the glmS ribozyme from Bacillus subtilis for gene silencing in T. brucei, which has been shown to control reporter gene expression in response to exogenous glucosamine. In this work we used the CRISPR/Cas9 system for endogenously tagging T. cruzi glycoprotein 72 (TcGP72) and vacuolar proton pyrophosphatase (TcVP1) with the active (glmS) or inactive (M9) ribozyme. Gene tagging was confirmed by PCR and protein downregulation was verified by real time PCR and western blot analyses. Further phenotypic characterization was performed by immunofluorescence analysis and quantification of growth in vitro. Our results indicate that the method was successful in silencing both genes without the need to add glucosamine to the medium, suggesting that T. cruzi produces enough levels of endogenous glucosamine-6-phosphate to stimulate the glmS ribozyme activity under normal growth conditions. This method could be useful to obtain knockdowns of essential genes in T. cruzi and to validate potential drug targets in this parasite.

81 A targeted RNAi screen identifies genes that play a role at different stages of Trypanosoma brucei metacyclogenesis.

Nkouawa, Agathe Y. Toh, Justin; Rojas-Sánchez, Saúl; Shi, Huafang; Lei, Yuling; G. Kolev, Nikolay; Tschudi, Christian (Yale School of Public Health)

By overexpressing a single RNA-binding protein, RBP6, in non-infectious procyclics trypanosomes, we previously recapitulated in vitro the events leading to acquisition of infectivity in tsetse flies. To begin to establish a roadmap for this developmental progression, we selectively targeted genes that were up- or down-regulated in both differentiating parasites or purified metacyclics relative to uninduced procyclics, and genes involved in the transition from slender bloodstream to stumpy forms. We generated 74 inducible RNAi cell lines in the background of inducible RBP6 expression and analyzed the RNAi effect on BARP and metacyclic VSG expression, as well as kinetoplast repositioning. Our screen discovered 24 genes that affected metacyclogenesis at different stages. RNAi against 6 genes, ring finger domain containing protein (RING1), phosphatase and tensin homolog (Ph290), dual-specificity phosphatase (DSPhos), protein kinase A-regulatory subunit (PKA-R), cold shock domain protein 2 (CSD2), and Puf11 inhibited epimastigote kinetoplast repositioning and RNAi targetting AMP-activated protein kinase a2 subunit (AMPKa2) or ZC3H45 stimulated kinetoplast relocation relative to the parental strain. BARP expression was decreased by at least 50% in RNAi cell lines against 21 genes, AGC/RSK serine/threonine kinase, cyclin 8, Wee1-1 like kinase, CMGC/DYRK protein kinase, GPI-anchor transamidase subunit 8, hypothetical protein 2, RING1, Ph290, DSPhos, AMPKa2, AMPKß, AMPK?, PKA-R, CSD1, CSD2, DRBD6A, DRBD6B, DRBD11, Puf11, and ZC3H45. Finally, RNAi against 20 genes resulted in at least 50% decrease in mVSG397 expression. The identified genes were Ph290, DSPhos, PIP39, AMPKa2, AMPKß, AMPK?, PKA-R, a rac-serine kinase, CSD1, CSD2, DRBD6A, DRBD6B, DRBD11, Puf11, RBP3, ZC3H45, GPI8, Hyp2, metacaspase 1 and RING1. RBP7A appeared to be a negative regulator of mVSG expression, since RBP7A RNAi increased the expression of metacylic VSGs. Intriguingly, previously identified components of the pathway regulating differentiation from stumpy bloodstream to procyclic forms, like PTP1 and PIP39, are also involved in regulating the process of metacyclogenesis.

82 Activity of fire ant venom alkaloids against Trypanosoma cruzi: quest for an effective alternative therapy

Heise, Norton Costa Silva, Rafael; Fox, Eduardo (Universidade Federal do Rio de Janeiro); Gomes, Fabio (NIAID-NIH); Feijó, Daniel; Ramos, Isabela (Universidade Federal do Rio de Janeiro); Koeller, Carolina (SUNY - University at Buffalo); Costa, Tatiana; Rodrigues, Nathalia; Lima, Ana Paula; Atella, Georgia; Miranda, Kildare (Universidade Federal do Rio de Janeiro); Schoijet, Alejandra; Alonso, Guillermo (INGEBI-CONICET); Machado, Ednildo (Universidade Federal do Rio de Janeiro)

Toxic effects of solenopsin alkaloids extracted from the venom of two species of fire ants were evaluated against the protozoan parasite Trypanosoma cruzi, etiologic agent of Chagas disease. IC50 determinations showed that solenopsins are 20 times more active against epimastigotes of two T. cruzi strains when compared to benzonidazole, the drug of choice for Chagas disease treatment, and presented a selective index of 5 when tested against different cultured mammalian cells. Fluorescent intercalator displacement assays indicate that solenopsins do not interact with DNA. Parasites become swollen and rounded in shape after treatment, with hypertrophied contractile vacuoles, elevated long-chain polyphosphate levels and intense cytoplasmic vacuolization possibly as a result of osmotic stress, but showing no accumulation of multiple kinetoplasts and/or nuclei. Overexpression of phosphatidylinositol 3-kinase - essential for osmoregulation and a target of solenopsins in mammalian cells - did not prevent swelling and vacuolization nor counteracted effects of the alkaloids on parasites growth. Additional experimental approaches suggested that solenopsins induce an autophagic (based on increased (i) number of concentric membrane structures similar to autophagosome-like bodies, (ii) monodansylcadaverine labeling, (iii) expression of the Atg8 autophagosomal membrane marker) and apoptotic (based on increased DNA fragmentation through specific generation of free 3'-OH ends that was not observed in untreated controls or TX-100 treated parasites) process in T. cruzi. Finally, solenopsins reduced proliferation of intracellular amastigotes from infected macrophages in a concentration-dependent manner, and were toxic against Leishmania donovani promastigotes and Trypanosoma brucei rhodesiense bloodstream forms, two other important human harmful kinetoplastid parasites. The results suggest solenopsins as novel platforms for development of new drugs against neglected diseases caused by different kinetoplastids.

83 Addressing the choices made in VSG switching by single cell RNA sequencing

Subota, Ines Wange, Lucas; Brink, Benedikt; Luzak, Vanessa; Enard, Wolfgang; Siegel, Nicolai (LMU Munich)

Trypanosoma brucei cells exchange their surface protein coat as a means to escape the host immune response. This event is stochastic in every parasite cell and the choice is taken out of a minimum of 2000 possibilities. The underlying mechanism is either transcriptional or recombinational and can be more or less frequent depending on the strain. VSG frequencies at given times were studied at the population level (in vivo and in vitro), revealing a certain hierarchical order in the complexity. We will make use of recent advances in single cell RNA sequencing as a potent tool to answer the question if there is a pattern in the choice of VSG switching in single cells.Switching frequency in laboratory-adapted strains is extremely low, so we applied the CRISPR/Cas9 system to sequence-specifically target the transcribed VSG2 gene and introduced double strand breaks within. While a lot of cells die, we observed the emergence of a population that is purely negative for VSG2 by flow cytometry, several days after the induction of the cutting event. We now analyze the switching events in single cells with the single cell RNA barcoding and sequencing (SCRB-seq) method combined with molecular crowding (mcSCRB-seq). With reduced costs and similar sensitivity compared to other recent techniques we aim at deciphering the progression in VSG expression and type of switching events in a high number of single cells.

84 Analysis of molecules released by African trypanosomes and their role in parasite transmission

Tettey, Mabel Deladem Rojas, Federico; Matthews, Keith R. (School of Biological Sciences, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland)

Molecules released by African trypanosomes have an identified role in parasite virulence and quorum sensing, and may also be important at various stages of the parasite life-cycle. Some of these secreted proteins include peptidases, with functions in creating the density-sensing signal in the bloodstream (e.g. Rojas et al., 2019) and potentially interacting with the mammalian immune system or during infectivity of the tsetse fly. We, therefore, set out to identify proteins secreted by bloodstream forms (slender and stumpy) of Trypanosoma brucei and during defined windows of differentiation from stumpy to procyclic cells. Using detailed mass spectrometric analysis of the T. brucei secretome, we have identified 311 proteins to be released by the slender forms and 330 by the stumpy forms, with nine peptidases being significantly enriched in the stumpy forms. Samples were also analysed at three, six and eight hours during synchronous differentiation from stumpy to procyclic forms and five peptidases were identified to be strongly enriched at three hours into the differentiation process. These five peptidases may be associated with either differentiation or early colonization in the tsetse fly. Using a combination of CRISPR/CAS9 mediated knock-outs and endogenous gene tagging in pleomorphic T. brucei of the nine peptidases upregulated in stumpy forms, we have validated the secretion of these peptidases in stumpy forms and begun the exploration of their functions in differentiation and/or interaction with the immune system of the host, or early in transmission.Oligopeptide Signaling through TbGPR89 Drives Trypanosome Quorum Sensing.Rojas F, Silvester E, Young J, Milne R, Tettey M, Houston DR, Walkinshaw MD, Pérez-Pi I, Auer M, Denton H, Smith TK, Thompson J, Matthews KR. Cell. 2019

85 Analyzing the basic function of Trypanosoma brucei's two introns

Srivastava, Ankita O'Connor, Zachary; Günzl, Arthur (UConn Health)

In Trypanosoma brucei, all mRNAs are trans-spliced whereas there are only two introns, disrupting the coding regions of poly(A) polymerase 1 (PAP1, Tb927.3.3160) and of the putative DEAD-box RNA helicase DBP2B (Tb927.8.1510). A survey of kinetoplastid genomes revealed that every species harbors orthologous PAP1 and DBP2B genes with introns in the exact same position. Since the introns are also present in Bodo saltans, it appears that they withstood genome streamlining in trypanosomes for approximately 500 million years of evolutionary time. PAP1 is essential and functions in snoRNA biogenesis (Chikne et al., 2017, J Mol Biol 429:3301-3318). Similarly, DBP2B silencing halted culture growth within 3 days. Given the importance of the two genes and the long-term positional conservation of their introns, we hypothesize that these introns fulfill fundamentally important functions.We have started to investigate the function of the PAP1 intron. First, to investigate effects of intron deletion, we functionally fused PAP1 at its N-terminus with the PTP tag in procyclic trypanosomes, allowing us to specifically monitor expression from the manipulated PTP-PAP1 allele. Across different cell lines, intron deletion resulted in a ~2.5 fold increase of both mature mRNA and protein. Interestingly, our data indicate that trypanosomes harbor a nuclear reservoir of PAP1 pre-mRNA that is correctly end-processed but retains the intron. This pre-mRNA population seems to be important for trypanosomes because a cell line in which both PAP1 introns were deleted, exhibited a growth defect. Another unexpected observation was that silencing of DPB2B affected PAP1 pre-mRNA cis but not trans splicing, indicating that PAP1 expression is regulated by its intron, possibly with DPB2B in a direct role.

86 Building a Genetic Toolkit for Crithidia fasciculata

Malfara, Madeline DiMaio, John; Povelones, Megan (Penn State Brandywine)

Crithidia fasciculata is a model kinetoplastid that is particularly useful in biochemical experiments because it can reach high densities. When the first kinetoplastid genomes were sequenced, pathogenic species were given priority, leading to the development of an array of genetic tools in these organisms. Now, with the sequencing of the C. fasciculata genome, we are revisiting this organism as a model for kinetoplastid biology. C. fasciculata maintains episomal plasmids, which we have used to create cell lines that express cytoplasmic and mitochondrial GFP. We have also successfully targeted GFP to the tubulin locus by assembling a plasmid using a one-step, 4-way ligation protocol, which was developed by the Yates lab for use in Leishmania. We have also used this procedure to make constructs for gene knockout and protein destabilization. Introduction of these constructs into C. fasciculata has been challenging. Therefore, we have used a plasmid developed in the Gluenz lab to create a C. fasciculata cell line that expresses Cas9 and T7. We are now targeting genes for tagging or knockout using CRISPR/Cas9-mediated cleavage. By using this technique, we hope to allow for a more high-throughput analysis of gene function in C. fasciculata.

87 Characterization of Deubiquitinases involved in endocytic pathway in T. cruzi

SOUZA-MELO, NORMANDA BRITO SILVA, NATHALIA; PEDROSO SANTOS, GREGORY (FEDERAL UNIVERSITY OF SAO PAULO); MARIA ALCANTARA, LAURA (UNIVERSITY OF SAO PAULO); MUNIZ MALVEZZI, AMARANTA; SCHENKMAN, SERGIO (FEDERAL UNIVERSITY OF SAO PAULO)

Trypanosoma cruzi has a life cycle complex alternating between non-replicative and replicative forms. Replicative epimastigotes have high endocytic activity, which is decreased during the transformation to infective and non-replicative T. cruzi trypomastigotes. In epimastigotes, endocytosis occurs thought the flagellar pocket or the cytostome. The incorporated cargo is transferred initially to early endosomes and then is delivered to terminal secondary endosomes, also called reservosomes, an organelle rich in hydrolases. In Trypanosoma brucei, knockdown of deubiquitinases USP7 and Vdu were previously found to prevent suramin toxicity by affecting transference of this cargo to secondary lysosomes. As similar DUBs are detected in Trypanosoma cruzi genome, we hypothesized that the orthologous proteins (TcUSP7 and TcVdu) could also regulate the different endocytic process during differentiation of T. cruzi. By using GFP fusions, we found that both proteins localize in the parasite cytosol with patterns similar to the localization observed for T. brucei. TcVdu::GFP, but not TcUSP7::GFP overexpressors presented decreased cell infectivity and delayed parasite egress from the host cell. It was not possible to obtain TcUSP7KO using different approaches, suggesting that it is an essential gene. In contrast TcDUBs knockdown was generated by using CRISPR/Cas9 system. The resulting cell line showed changes in the delivery of transferrin to reservosomes and different distribution of cruzipain containing organelles, indicating its participation in the endocytosis pathway of T. cruzi.

88 CHARACTERIZATION OF THE DNA DAMAGE RESPONSE KINASES ATR AND ATM IN LEISHMANIA MAJOR

Almeida da Silva, Gabriel Lamak Bastos, Matheus S; Virgilio, Stela; Black, Jennifer Ann; McCulloch, Richard; Orsini Tosi, Luiz Ricardo

ATR and ATM, members of the phosphatidylinositol 3-kinase-like family, are master regulators of the eukaryotic response to DNA injuries. Their concerted action orchestrates cell cycle arrest, replication fork stabilization and DNA repair recruitment, which altogether provide genome maintenance and stability. Leishmania parasites have an unusually plastic genome characterized by gene and chromosome copy number variation, mosaic aneuploidy and chromosome rearrangements, which are frequent hallmarks of genome instability. Thus, we sought to investigate the conservation and functional relevance of ATR and ATM in the DNA metabolism of Leishmania major. We used CRIPSR/cas9 genome editing to generate ATR- and ATM-deficient cell lines. We were unable to select an ATR-null mutant, which suggests this is an essential gene in Leishmania. In a further attempt, we aimed to edit out only the last third of the ATR gene, which encodes the kinase domain, and selected a heterozygous cell line bearing both the truncated and an intact copy of ATR. Cell cycle progression and growth of these ATR-deficient cells are unaffected under normal conditions, but their response to Hydroxyurea-mediated replication stress is significantly different when compared to wild type cells. This is also observed when measuring the accumulation of phosphorylated histone H2A,, a DNA damage marker in trypanosomatids, which was drastically decreased in these cells. Different form ATR, an ATM-null mutant was promptly selected, despite the cells showing a marked effect on parasite growth even in the absence of DNA damage agents. Further characterization of the ATR and ATM-deficient cell lines will be presented. Supported by: FAPESP- 2016/16454-9. Keywords: Leishmania; DNA damage response; ATR kinase; ATM kinase; genome stability.

89 Characterization of wild trypanosome coats using spliced-leader sequence enrichment and RNA-Seq

So, Jaime Otieku Oworae, Kwadwo; Manful Gwira, Theresa (University of Ghana); Mugnier, Monica (Johns Hopkins Bloomberg School of Public Health)

African Trypanosomes defend themselves against host antibody in the bloodstream and tissue through antigenic variation of a highly immunogenic coat of variant surface glycoproteins (VSG). These parasites persist in the host by utilizing a large genomic repertoire of VSG genes and pseudogenes to switch the expression of their surface coats throughout infection. Studies of VSG expression in Trypanosoma brucei have revealed much of the current knowledge regarding antigenic variation in African trypanosomes. However, recent genetic analyses of Trypanosoma vivax suggest potential differences in this species that could affect antigenic variation, including a lower capacity for recombination and decreased density of the VSG coat. To better understand the in vivo VSG expression dynamics of T. vivax, we analyzed the transcriptomes of parasites in blood samples collected from naturally infected cows living in Bolgatanga and Adidome, which are located in northern and southern Ghana respectively. RNA was extracted from whole host blood which was collected four times from each cow over a monitoring period of six months. The study of gene expression in wild trypanosome populations is particularly difficult due to the low parasitemia of natural infections; RNA isolated from whole blood or tissue contains a very small fraction of trypanosome RNA in a mixture of host RNA. To overcome this problem, we developed an RNA-seq library preparation protocol which jointly depletes ribosomal RNA and host messenger RNAs. This protocol takes advantage of the 5' spliced-leader (SL) sequence present on mature mRNAs in all trypanosomatid species. Using a biotin-streptavidin pull-down technique, we achieve specific enrichment of parasite transcripts from whole host blood RNA samples. Our parasite-enriched transcriptomes allow us to characterize the VSG expression profile and identify other highly expressed putative surface proteins of Trypanosoma vivax across geographically and temporally distinct natural infections. This SL enrichment technique can easily be adapted and applied to in vivo studies for any the African trypanosome species by using each of their unique SL sequences as biotinylated baits.

90 Controlling the surface proteome: A novel nndosomal tetratricopeptide-repeat protein interacts with deubiquitylase TbUsp7 and the TbSkp1-like protein in Trypanosoma brucei.

Yamada, Kayo Canavante, Ricardo; Zoltner, Martin; Field, Mark (University of Dundee)

S-phase kinase-associated protein 1 (SKP1) is plays a crucial role in cell cycle progression, transcriptional regulation, signal transduction, and many other cellular processes by virtue of being a component of Cullen E3 ubiquitin ligases. Phylogenetic analysis revealed that Trypanosoma brucei possesses at least three Skp-1/Elongin like proteins. Two deubiquitilating enzymes (DUBs), TbUsp7 and TbVdu1, control the abundance of the invariant surface proteins ISG75 and ISG65. ISG75 trafficking is key to suramin uptake and TbUsp7 silencing partially blocked endocytosis. Using untargeted proteomics, we find that the Skp1-like protein of trypanosomes, Tb927.10.11610, decreases in abundance following TbUsp7 knock down and physically interacts with TbUsp7 and a tetratricopeptide-repeat (TPR) protein (Tb927.11.810) which is located close to the flagellar pocket/endosomes, indicating a likely role in endocytosis. Tb927.11.810 is also implicated in suramin sensitivity. Further, silencing Tb927.10.11610 reduced the abundance of TbUsp7 and Tb927.11.810 TPR protein. Taken together these data suggest that Tb927.11.810 is a novel component of the endosomal system that has specific responsibilities for coordinating ubiquitylation of surface proteins.

91 Damage and Antigenic Variation in African trypanosomes.

McLaughlin, Emilia Chaze, Thibault; Matondo, Mariette (Institut Pasteur Paris); Urbaniak, Michael (Lancaster University, UK); Glover, Lucy (Institut Pasteur Paris)

Trypanosoma brucei evades the host immune system through stochastic switching of the variant surface glycoprotein (VSG) expressed at the parasite cell surface. The single active VSG is expressed from a subtelomeric Expression Site (ES) and DNA double-strand breaks (DSBs) in the subtelomeric ESs are triggers for VSG switching. To determine the cellular response to a DSB at the active ES we are using quantitative phosophoproteomics. Using an inducible meganuclease-based system (Glover et al., 2008, 2013) to introduce a DSB at either a chromosome-internal locus or within the active ES, we can compare the resulting phosphoproteomes to reveal factors specifically phosphorylated at the active ES and thus required for antigenic variation. A DSB at the active ES results in approximately 95% cell death (Glover et al., 2013). In order to investigate the cause of the DSB lethality, we expressed a second VSG, VSG5, from a ribosomal locus. Interestingly, the VSG5 gene is lost following a DSB at the active ES, with no change to cell survival. We now aim to introduce VSG5 into the pseudo gene of the active ES in order to determine whether this provides a survival advantage upon break induction. Combined, our work aims to identify factors required for VSG switching, allowing T. brucei to evade the host immune system.

92 Depleting extracellular Ca2+ leads to a decrease in cytotoxicity due to enhanced Trypanosome-protective variants of APOL1

Khalizova, Nailya Shirinyan-Tuka, Lilit; Giovinazzo, Joe; Thomson, Russell; Raper, Jayne (Hunter College CUNY)

Trypanosomes are a major agricultural barrier in sub-Saharan Africa, affecting livestock. Humans, however, are immune to some species of trypanosomes due to immunity conferred by an innate immunity protein, APOLIPOPROTEIN L1 (APOL1). Recently evolved variants of APOL1 in humans are linked to an increased chance of developing kidney disease. While the trafficking of APOL1 in trypanosomes has been widely characterized, the pathway of APOL1 toxicity in the kidney is not clear. To elucidate APOL1's role in increased kidney toxicity, we looked at the trafficking of APOL1 in a mammalian cell system. Using live wield-field fluorescent microscopy, we previously showed that APOL1 forms Ca2+ conductive channels at the surface of mammalian cells. In order to elucidate the role of Ca2+ conductance in APOL1 toxicity, we have been measuring cell death induced by APOL1 in cells that were treated with different amounts of EGTA, a Ca2+ chelator. Based on the results so far, reducing extracellular Ca2+ levels with 1.5 mM EGTA leads to ~25% reduction in toxicity due to kidney disease variants in-vitro. Currently, we are working on confirming our results and further elucidating the role of Ca2+ conductance in APOL1 toxicity. Eventually, understanding the role of Ca2+ in APOL1 cell toxicity pathway could be useful for targeted drug development in the future.

93 Detection of Trypanosoma cruzi Secreted Antigens in Blood of Infected Hosts

Acosta, David Nagarkatti, Rana (CBER, Food and Drug Administration); Fortes de Araujo, Fernanda; Teixeira-Carvalho, Andréa (Instituto René Rachou, Fiocruz); Debrabant, Alain (CBER, Food and Drug Administration))

Chagas drug discovery has been hampered by a lack of validated assays to establish treatment efficacy in pre-clinical animal models and in patients infected with T. cruzi. Serological and PCR based assays are not reliable to demonstrate reduction in parasite burden particularly in the chronic phase of Chagas disease. However, reduced levels of parasite secreted antigens in the blood of infected hosts could be used to demonstrate treatment efficacy. A published proteomic study of parasite secreted antigens identified the hypothetical protein TCSYLVIO_5171 as a secreted antigen. We expressed and purified TCSYLVIO_5171 as a recombinant protein in E. coli and generated rabbit polyclonal antibodies against the full-length protein and against an immunodominant peptide epitope. Immuno-fluorescence assays showed that the native protein was expressed in the cytoplasm of the three life cycle stages of the parasite. The molecular weight of this antigen was estimated to be ~30 kDa by western-blot analysis of parasite lysates. Anti-peptide antibodies were able to detect the parasite antigen in blood of infected mice during the acute and the chronic phase of infection. Benznidazole treatment significantly reduced the antigen levels in treated mice, however, all the mice remained PCR positive indicating reduction in parasitemia but not cure. In patients from Brazil diagnosed with indeterminate Chagas disease, TCSYLVIO_5171 antigen levels were significantly higher compared to endemic controls. Pair-wise analysis of patients, before and after benznidazole treatment, showed a significant reduction in antigen levels post treatment. The antigen was also detected in patients with cardiac Chagas disease. Taken together, our results indicate that TCSYLVIO_5171 could be used as a biomarker of Chagas disease and to assess treatment efficacy.

94 Development of the protein kinase, AEK1, in Trypanosoma brucei as a promising drug target

Jensen, Bryan Parsons, Ben; Booster, Nicholas; Dean, Marissa (Seattle Children's Research Institute); Vidadala, Rao; Maly, Dustin (University of Washington); Parsons, Marilyn (Seattle Childrens Research Institute)

Protein kinases (PKs) have proven to be exceptional targets for chemotherapeutic intervention for cancer and other diseases in humans. We want to exploit the wealth of resources developed for treating these diseases as starting points in the efforts to develop therapeutic agents against T. brucei. We began our search for good drug targets with a list of essential kinases as determined by RNAi [Jones el al, PMID:24453978]. We used a conditional knockout to verify one PK (AEK1, Tb927.3.2440) is essential. We demonstrated that the kinase activity of AEK1 is essential by generating analog-sensitive (AS) mutations in AEK1. PKs with AS-mutations site can bind bulky ATP-analogs (BKIs), which function as specific inhibitors. Expression of AS-AEK1rescued the conditional knockout but rendered growth of the strains sensitive to the BKI both in vitro and in vivo. In contrast strains expressing wild-type AEK1 are significantly less sensitive to the BKI. These data provide chemical validation of AEK1. We are currently screening a collection of small, drug-like inhibitors developed against other protein kinases for binding to AEK1 as a starting point for drug discovery.The closest mammalian homologs to AEK1 are the AKT family of PKs. We tested conserved motifs between AEK1 and the AKT protein involved in regulation by testing the ability of mutant AEK1s to rescue the cKO. A C-terminal hydrophobic motif essential for AKT1. To be fully active AKT requires phosphorylation of three residues and phosphoproteomic analysis identified phosphorylation of similar residues in AEK1 (TriTrypDB). We have confirmed the importance of these residues for the function of AEK1. The kinases responsible for these modifications may provide additional targets for drug discovery.

95 Developmental Regulation of CYb and COIII mRNA Editing Occurs by Distinct Mechanisms in Trypanosoma brucei

Smith Jr., Joseph Tylec, Brianna; Read, Laurie

The majority of mRNAs encoded in T. brucei mitochondrial maxicircles require the insertion/deletion of uridylate residues to achieve translatable open reading frames. Precise editing is directed by guide RNAs (gRNAs) encoded in the DNA minicircles. Historical data indicate that some mRNAs are only fully edited in procyclic form (PCF) but not in bloodstream form (BSF). Here, using updated methods, we investigated the editing of three mRNAs: CYb, COIII, and A6. Total levels of all three mRNAs are reduced in BSF as shown by qRT-PCR. Fully edited CYb and COIII mRNAs are undetectable in BSF, while some edited A6 is present. High throughput sequencing showed that the CYb mRNA population in BSF is almost entirely pre-edited. Combined with qRT-PCR findings, our data indicate that the amount of pre-edited CYb mRNA stays relatively stable in both life cycle stages, and suggest that CYb mRNAs that enter the editing pathway become rapidly degraded. In contrast, the majority of A6 and COIII mRNAs are partially edited in PCF and BSF. We determined the sites at which A6 and COIII mRNA editing intrinsically pauses in both life cycle stages and further analyzed the most abundant junction sequences upstream of these pause sites. Altogether, we found that although the vast majority of COIII mRNA does enter into the editing pathway in BSF, editing is significantly interrupted within the region of the first 3 guide RNAs. This contrasts with A6 mRNA, in which productive editing still progresses well past the region of the first 3 guide RNAs in both BSF and PCF. Further studies are needed to determine how BSF parasites inhibit the editing of COIII mRNA.

96 Dissecting the Functions of a Divergent Mitochondrial DNA Polymerase in Trypanosoma brucei

Delzell, Stephanie Nelson, Scott (Iowa State University); Klingbeil, Michele (University of Massachusetts, Amherst)

Mitochondrial DNA structure and replication in trypanosomes are essential for parasite survival and highly divergent from those in animal cells. Unlike most eukaryotes, which have one essential replicative polymerase, T. brucei has three DNA polymerases that are essential for kDNA replication. TbPOLIB and TbPOLID are essential for minicircle replication, although neither protein has been enzymatically characterized to evaluate whether it is a high fidelity enzyme or playing a supporting role. Predictive modeling and homology alignments of TbPOLIB revealed the archetypical right hand structure, but also structural features making this protein unique among all known Family A DNA polymerases. One predicted feature is a large thumb domain insertion (369 amino acids) with homology to RNase T, in addition to the presence of a more canonical exonuclease domain. To understand how unique structural features contribute to TbPOLIB activity and its role in kDNA replication, we are characterizing the enzymatic activities using recombinant protein. Due to previous challenges expressing the full-length protein, a codon-optimized truncated version of TbPOLIB based on the homology model was designed and expressed as with a His tag in E. coli. Affinity purification of two variants confirmed nucleotidyl incorporation for WTtrunc TbPOLIB, and loss of activity in a polymerase deficient mutant (Pol-) in a standard primer extension assay using activated calf thymus DNA. Following further purification using an anion exchange column, WTtrunc TbPOLIB demonstrated robust exonuclease activity on fluorescently labeled DNA visualized on a denaturing gel that outcompeted nucleotidyl incorporation. Completion of the first detailed enzymatic characterization of an essential kDNA polymerase will provide the foundational information necessary for future high-throughput screening, enabling the identification of inhibitors of TbPOLIB.

97 DNA Damage Response in African Trypanosomes

Sima, Nuria Coron, Ross; Glover, Lucy (Institut Pasteur)

Antigenic variation in Trypanosoma brucei (T. brucei) is triggered by DNA double strand breaks (DSBs) at the active telomeric Expression Site (ES), subsequent repair by homologous recombination (HR) allows for variant surface glycoprotein (VSG) exchange and switching. Although this process is essential for antigenic variation and immune evasion, relatively little is known about the DNA damage response at this locus. Using an inducible I-SceI meganuclease-based system, single DSBs have been generated in telomeric and non-telomeric loci and the focal accumulation of phosphorylated histone H2A (gH2A), replication protein A (RPA), BRCA2 and RAD51 recombinase assessed (Glover et al., 2008 Nulceic Acids Res; 2013 PLoS Pathog). We now aim to characterize the factors required for DSB repair at both a chromosomal internal locus and telomeric VSG ES. Using endogenous tagging of the BRCA2 protein coupled with immunoprecipitation-mass spectrometry (IP-MS) we can identify interacting, and potentially novel, proteins and protein complexes required for repair. We are also developing an endonuclease-deactivated Cas9 (dCas9) system to purify and mark discrete regions of chromatin which will allow us to study DNA repair dynamics, in an effort to better understand the role of DNA repair and recombination in antigenic variation.

98 DNA Replication in Leishmania – an odd start?

Marques, Catarina Damasceno, Jeziel; McCulloch, Richard (Wellcome Centre for Integrative Parasitology, University of Glasgow)

In eukaryotes, DNA replication is initiated at multiple sites (origins of replication) across each chromosome, whereas in all studied bacteria, and many archaea, it is initiated at a single origin. How origin multiplicity and associated controls evolved is unknown, but recent findings suggest that investigating this process in Leishmania may provide insight. DNA replication sites in L. major have been recently mapped using two different genome-wide methods with apparent conflicting results: while MFA-seq detects a single origin per chromosome, SNS-seq results indicate thousands of initiation sites across the genome. We propose that the data from both studies can be reconciled and explained by a bimodal strategy of DNA replication in Leishmania, where a single conventional origin per chromosome is activated in every cell cycle (mapped by MFA-seq), while additional, origin-independent initiation events occur stochastically throughout the genome (detected by SNS-seq), thereby ensuring complete genome replication. We further suggest that these origin-independent initiation events are triggered by DNA recombination, possibly explaining Leishmania's pervasive genome plasticity. To test this hypothesis, we will describe the use of a combination of inducible DiCre and CRISPR/Cas9 technologies to rapidly and specifically generate inducible mutants of constituents of the origin recognition complex and the replicative helicase in L. major. We will describe the effect of these ablations on growth, DNA replication and genome stability. Using the same approach, we will epitope tag components of the two complexes, allowing is to examine protein expression and map genome localisation.

99 Expression of mutant variant surface glycoprotein in bloodstream form Trypanosoma brucei triggers a severe growth defect

Ooi, Cher-Pheng Schwede, Angela; Carrington, Mark (Cambridge University); Rudenko, Gloria (Imperial College)

Variant surface glycoprotein (VSG) is the most highly expressed protein in bloodstream form (BSF) Trypanosoma brucei. VSG constitutes 10% of total cellular protein and ten million GPI-anchored VSGs coat the cell surface. VSG expression is essential in BSF T. brucei and blocking VSG synthesis using RNAi or synthetic Morpholinos causes an abrupt cell cycle arrest where cells duplicate their kinetoplast and nucleus (2K2N) but do not initiate cytokinesis. Given its essentiality for cell viability, does perturbing VSG expression in other ways trigger a stress response in BSF T. brucei? Knockdown of the GPI-transamidase GPI8 impairs GPI addition to all anchored surface proteins. We show that GPI8 RNAi results in growth arrested cells with an enriched 2K2N population morphologically similar to cells blocked for VSG synthesis. These pre-cytokinesis (2K2N) cells are less abundant (25 – 30%) compared to when VSG synthesis is blocked (60%). This growth arrest is likely triggered by the clogging of the endoplasmic reticulum (ER) by unanchored surface proteins. As VSG constitutes 90% of cargo trafficked through the ER, we generated cells inducible to express an ectopic mutant VSG with disrupted intramolecular disulphide bridges. When mutant VSG is expressed, cells rapidly undergo a severe growth defect and the population is enriched for 2K2N cells (25 – 30% of cellular population) morphologically similar to cells blocked for VSG synthesis. This growth defect is reversible up to 48h after induction by washing out the inducing drug. These data suggests that BSF T. brucei triggers a stress response when expressing mutant VSG. This possibly also occurs during VSG switching after a gene conversion event which results in the expression of a defective VSG.

100 Fatty acid synthesis and lipid homeostasis in T. brucei: a response to ACC depletion

Saliutama, Joshua Vigueira, Patrick; Paul, Kimberly (Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University)

T. brucei utilizes an unconventional fatty acid elongation pathway for de novo fatty acid synthesis. Insect procyclic forms (PFs) synthesize a range of fatty acid chain lengths, while bloodstream forms (BFs) make primarily myristate (C14:0), which is the fatty acid used for GPI-anchoring of Variable Surface Glycoprotein (VSG), the primary surface coat protein antigen. This fatty acid synthesis requires the two-carbon donor malonyl-CoA, which is synthesized by Acetyl-CoA Carboxylase (ACC). Our lab generated a knockdown cell line that reduces ACC transcripts through RNA interference (RNAi). Despite a >90% reduction in ACC protein, ACC RNAi did not affect growth in culture, suggesting that BF T. brucei can compensate for reduced fatty acid synthesis. However, ACC RNAi cell lines showed reduced virulence in a mouse model of infection, suggesting that the compensation was not sufficient to support full virulence in vivo. To examine how T. brucei responds to the loss of ACC, we are performing global lipidomics analyses. Our preliminary lipidomics data indicated no change in cellular levels of medium and long-chain fatty acids, including myristate. However, the data did show changes in other lipid species, such as neutral lipids, lysophospholipids, and phospholipids. Altogether, these data show how membrane and storage lipids are remodeled in T. brucei in response to ACC-depletion and reduced fatty acid synthesis and may reveal lipid factors important for virulence.

102 Genetic tools for efficient inducible expression and high-complexity library production in Trypanosoma congolense

Awuah-Mensah, Georgina Steketee, Pieter; Morrison, Liam (University of Edinburgh); Gadelha, Catarina; Wickstead, Bill (University of Nottingham)

Trypanosoma congolense is a livestock-infective parasite of substantial economic importance. It is known to be biologically different from T. brucei in key aspects of metabolism, life-cycle control and drug response. It is also the only salivarian trypanosome whose complete life-cycle can be reiterated in culture without genetic manipulation. Differences from T. brucei plus the potential to address additional aspects of life-cycle control, create a pressing need for high efficiency genetic tools for T. congolense.Existing genetic tools to study T. congolense rely on heterologous use of T. brucei vectors and target loci unsuitable for tightly regulated expression. In addition, transfection efficiencies have restricted application, especially in bloodstream forms, excluding the possibility of creating libraries of transformants. Here, we describe a specific molecular toolbox for tightly-regulated inducible expression in bloodstream-form T. congolense, its application in RNA interference, and the development of a system for high-complexity library production.We first established a T. congolense line expressing tetracycline repressor and T7 RNA polymerase regulated by endogenous processing sequences. By testing 3 potentially silent loci (in 2 orientations) we demonstrate regulation at each one, but with differing clonal variation and 'leakiness' in the off-state. Selecting the most tightly-regulated site, we show knockdown of clathrin, tubulin and exogenous GFP using a modified RNAi construct. Finally, we describe modification of this locus to inducibly create double-strand breaks. Modification at this site shows little variation between clones and is stable to freeze/thawing. Moreover, an induction of this cell line (Tco-TTS) generates 10,000-25,000 independent transformants per transfection. We discuss progress in applying this in genome-wide RNA interference studies.

103 Glycosylation of transferrin receptor (TfR) in bloodstream form Trypanosoma brucei: steric constraints control GPI-glycan modification

Koeller, Carolina M. *Tiengwe, Calvin (Imperial College); Schwartz, Kevin J. (University of Wisconsin-Madison); Bangs, James D. (University at Buffalo)*

Transferrin receptor (TfR) of bloodstream form (BSF) Trypanosoma brucei is a heterodimer of GPI-anchored ESAG6 (E6) and soluble ESAG7 (E7). Mature E6 has five N-glycans, representing a mixture of oligomannose and unprocessed paucimannose structures [Mehlert et al., 2012, PLoSP 8:e1002618]. However, TfR has been showed to bind tomato lectin (TL), which binds linear repeats of N-acetyllactosamine (LacNAc) [Nolan et al., 1999, Cur. Biol. 9:1169], and biosynthesis experiments show transport dependent increases in the size of E6 [Schwartz et al., 2005 JCS 18:5499] suggesting post-ER glycan processing. We now investigate TfR glycosylation by pulse-chase in conjunction with N-glycanase (PNG) treatment and sequential lectin pulldowns. E6 increases ~5 kDa during maturation, and this increase is lost with removal of N-glycans. Mature E6, was reactive with both TL and Erythrina cristagalli lectin (ECL, terminal LacNAc) indicating the presence of LacNAc on paucimannose N-glycans. Minor GPI processing was observed, presumably by limited galactose addition. However, increasing the spacing between E6 protein and the GPI anchor (E6HP, +4-7 aa) increased the sizes of intact and de-N-glycosylated mature E6 ~12 and ~7 kDa, respectively; both species were reactive with TL and ECL. These results indicate addition of LacNAc repeats to both N-glycans and the GPI anchor. The latter result was confirmed with identical assays of a non-N-glycosylated, GPI anchored reporter, BiPNHP, which increases ~10 kDa and acquires TL/ECL reactivity during intracellular transport. Collectively, these results suggest that given the proper substrate, BSF trypanosomes are capable of modifying GPIs by addition of poly-LacNAc structures similar to that found in insect stage trypanosomes. Genetic approaches targeting glycosyltransferases involved in N- and GPI glycan modifications are underway.

* These authors contributed equally to this work

104 How does a trypanosome change its spots? Decrypting immune avoidance in human kinetoplastids

Warren, Felix Llwellyn, Martin; McCulloch, Richard; Burchmore, Richard (The University of Glasgow); Gadelha, Catarina (The University of Nottingham)

For a pathogen, avoiding the host immune system is crucial for its survival. Trypanosoma brucei have a well described process for host immune avoidance using variable surface glycoproteins (VSG), called antigenic variation. This process is thought to rely on transcriptional and recombinational mechanisms producing a huge diversity of VSG mRNA, which is largely dependent on the presence of VSG pseudogenes. How and where VSG pseudogenes recombine to form VSGs is unknown, and how VSG coat diversity reflects VSG mRNA diversity is unknown. More widely, if and how genomic changes infer surface protein rearrangements under immune pressures are not well defined in related species. Importantly, in Trypanosoma cruzi and Leishmania spp., strategies for immune evasion are much less clearly understood and any link between genomic, transcriptomic and surface proteome variation due to immune pressure has not been examined.Currently, methodologies establishing the drivers of the surface proteome dynamics for kinetoplastid parasites are lacking. Here we hope to develop systems for increased proteomic accuracy with a view to linking proteomic changes with transcriptional or post-transcriptional modifications and possible genomic rearrangements whilst under pressure from the host immune system. Using new techniques not previously undertaken in these parasites for elaborating their spatial proteomics, we hypothesise that following immune pressures, proteomic evasion strategies may be connected to genomic rearrangements of the kinetoplastids examined here. By adding to the current knowledge of proteomic developments in these parasites we hope to further inform basic biology and potential translational therapies.

POSTERS: Session C
Tuesday 7:00 pm

04/30/19

105 – TT5H Characterising Leishmania kinetochores with XL-BioID

Geoghegan, Vincent Jones, Nathaniel; Dowle, Adam; Larson, Tony; Mottram, Jeremy (University of York)

106 – TT5I Identifying novel factors associated with trypanosome DNA replication forks using nascent DNA proteomics

Rocha-Granados, Maria Bermudez, Yahaira; Dodard, Garvin (University of Massachusetts); Gunzl, Arthur (UConn Health); Klingbeil, Michele M. (University of Massachusetts, Amherst, Amherst, MA)

107 – TT6H An ORF-based whole-genome gain-of-function library for Trypanosoma brucei

Quinn, McKenzie Gomez, Stephanie (The George Washington University); Schultz, Danae (Harvey Mudd College); Kim, Hee-Sook (Cleveland State University); Hovel-Miner, Galdriel (The George Washington University)

108 – TT6I UTR-seq: hundreds of new regulatory 3'-untranslated regions in African trypanosomes

Trenaman, Anna Wall, Richard; Horn, David (University of Dundee)

109 – TT6K Characterization of putative chromo- and SET-domain transcription regulators in Trypanosoma brucei

Staneva, Desislava Carloni, Roberta; Auchynnikava, Tatsiana; Tong, Pin; Matthews, Keith; Allshire, Robin (The University of Edinburgh)

110 – TT6L Intriguing roles assigned to Leishmania eIF4E paralogs by structure-function studies and CRISPR-Cas 9-mediated knockout approach

Shapira, Michal Tupperwar, Nitin; Shrivastava, Rohit; Kamus-Elimeleh, Dikla; Orr, Irit (Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel); Meleppattu, Shimi; Leger-Abraham, Melissa (Microbiology Department, Blavatnik Institute, Harvard Medical School, Boston, MA); Wagner, Gerhard (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA)

111 Overexpression of a Trypanosoma cruzi mitochondrial ribonuclease affects cell morphology and differentiation

Zimmer, Sara Ramirez-Barrios, Roger (Department of Biomedical Sciences, University of Minnesota Medical School, Duluth campus, Duluth, Minnesota, USA.)

Abundance of some edited (mature) mitochondrial mRNAs encoding electron transport chain (ETC) components increases during culture starvation of Trypanosoma cruzi epimastigotes. However, ETC function measured by parasite oxygen consumption rate actually decreases during this restriction, and no obvious changes in nuclear-encoded ETC component expression occur. The functional role of this spike in editing of mitochondrial mRNAs is therefore mysterious. We hypothesized that increased stores of these mRNAs may be necessary for starvation-mediated differentiation to the metacyclic form, and aimed to test this by preventing increases in editing during starvation. Overexpression of the Trypanosoma brucei mitochondrial exoribonuclease TbRND results in depletion of the guide RNAs editing factors and depletion in edited mRNAs. We reasoned that overexpression of the T. cruzi homologue of TbRND (TcRND) would also negatively impact editing, and could be a tool to determine functional effects of a lack of edited mitochondrial mRNAs. The gene encoding TcRND (TcCLB.510743.90) was integrated into the pTcINDEX construct and transfected into cells possessing pLEW13 (for T7 polymerase and tetR expression) for tetracycline-regulated expression of TcRND. “Leaky” TcRND overexpression was confirmed by qRT-PCR. TcRND overexpression in epimastigotes resulted in edited mRNA A6 (complex V component) and CO3 (complex IV component) depletion, albeit more modestly than in T. brucei. Importantly, differentiation to metacyclic trypomastigotes decreased ~60% in TcRND overexpressing cells. We also noted that while cell lengthening is a hallmark of glucose-starved epimastigotes “preadapted” for culture differentiation, epimastigotes overexpressing TcRND were shorter than the parent pLEW13 transfected cells and the original CL Brener strain. In summary, TcRND overexpression analysis suggests a link between mitochondrial mRNA abundances and cell morphology and differentiation capacity in T. cruzi.

112 Potential usage of extracellular amastigotes of Trypanosoma cruzi as a temporal axenic culture for transient gene expression, drug sensitivity assay, and CRISPR/Cas9-mediated gene knockout.

Takagi, Yuko Akutsu, Yukie; Doi, Motomichi; Furukawa, Koji

Mammalian stage of Trypanosoma cruzi, amastigote, is generally considered as an obligate intracellular parasite. However, in our hand, amastigote can proliferate in absence of the host cell at least for 1 week in LIT medium at 37°C. Taking advantage of this property, we utilized the axenic amastigote culture to explore its potential usage in various stage-specific studies. Conventional electroporation could introduce a plasmid to express exogenous gene in extracellular amastigote. The transfectant was selectable with a drug resistant marker to enrich the transformed population to certain extent, within the given time limitation of axenic culturing. Extracellular amastigote could also be used in drug titration assay. EC50s of Benznidazole and Nifurtimox for extracellular amastigote were comparable to those for intracellular amastigote. Moreover, gene knockout was successfully performed using Cas9-expresssing amastigote and synthetic gRNA. Growth phenotype of the knockout amastigote could be monitored either by continuation of axenic culturing or by host cell invasion followed by intracellular proliferation. Our results indicate that temporal axenic amastigote culture provides a useful experimental means to manipulate amastigotes, which are otherwise inaccessible due to the presence of the host cell.

113 Rapid block of RNA splicing by chemical inhibition of analog-sensitive CRK9 in Trypanosoma brucei

Gosavi, Ujwala A.

Gene silencing of the cyclin-dependent kinase CRK9 in trypanosomes exhibited a block of spliced leader trans splicing of nuclear pre-mRNA and a loss of phosphorylation of RPB1, the largest subunit of RNA polymerase II.To decipher CRK9's role in gene expression, we generated cell lines that exclusively express C-terminally PTP-tagged, ATP analog-sensitive (AS) CRK9 in which the gatekeeper residue M438 was substituted with glycine (CRK9AS1-PTP). Treatment of these cells with 1-NM-PP1, a bulky N6-enlarged ATP analog, inhibited culture growth of CRK9AS1-PTP-expressing cells, causing them to round up and die within 48 hours, similar to but much more rapid than CRK9-silenced cells. The compound was tenfold more effective in CRK9AS1-PTP- (EC50 of 1.5 mM) than in CRK9WT-PTP-expressing cells, indicating that the inhibitor effectively blocked the activity of CRK9AS1-PTP. When treated with 10 µM of 1-NM-PP1, a block of trans and cis (intron removal) splicing became apparent after 5 and 60 minutes, respectively, whereas it took ~6 hours before the loss of RPB1 phosphorylation was detectable. These results strongly indicate that the RNA processing machinery requires continuous input from CRK9 to remain active. In contrast to our notion that unphosphorylated RPB1 is defective in transcription, RNA pol II-mediated synthesis of selected transcripts continued even at 24 hours of drug treatment, suggesting that most RPB1 phosphorylations are not essential for transcriptional activity per se.Since we found that purified CRK9AS1-PTP but not CRK9WT-PTP accepts bulky phenyl-ethyl ATP?S for thiophosphorylation, we are adopting a pull-down assay using an anti-thiophosphate ester antibody to identify CRK9 direct substrates.

114 Regulation of expression of the RNA-binding protein RBP10 in Trypanosoma brucei

Bishola, Tania LIU, BIN (ZMBH); TERRAO, MONICA (UNIKLINIK KOLN); CLAYTON, CHRISTINE (ZMBH, UNIVERSITY OF HEIDELBERG)

T. brucei RBP10 is expressed in growing bloodstream forms but not in stumpy or procyclic forms. It binds to the motif UA(U)6 in the 3' UTRs of procyclic-specific mRNAs and targets them for destruction. RPBP10 is required for bloodstream form gene survival, and its expression in procyclic forms enables the trypanosomes to grow as bloodstream forms. Correct developmental regulation of RBP10 is therefore critical for the parasite life cycle. We are now investigating how the expression of RBP10 itself is regulated.Using differentiation-competent trypanosomes, we integrated a reporter open reading frame in the RBP10 locus and found that the 7.3 kb RBP10 3'UTR conferred the expected regulation: reporter protein was high in bloodstream forms, but undetectable in procyclic forms. The mRNA was also regulated, to a somewhat lesser extent. To identify the regions responsible, we tested progressively shorter segments of the 3'UTR for regulatory properties in comparison with the control actin 3'-UTR. The results indicate that regulatory sequences are scattered throughout the RBP10 3'-UTR. Some of them repress mRNA levels and translation in procyclics, while others do the opposite in bloodstream forms. Comparison of the regulatory segments with 3'-UTRs that give similar regulation should enable us to identify shared motifs.

115 RNA-binding protein 5 (RBP5) plays a role in cell cycle progression in Trypanosoma brucei

Anaguano, David Klingbeil, Michele M. (University of Massachusetts, Amherst, Amherst, MA)

Kinetoplastid parasites rely mainly on post-transcriptional regulation where RNA-binding proteins (RBP) have shown to play a major role by modulating the stability and translation of specific mRNAs. In Trypanosoma brucei, RBP6 and RBP10 are proteins that play crucial roles in procyclic and bloodstream form parasites differentiation, respectively. Overexpression of RBP6 stimulates differentiation of procyclics to the infectious metacyclic form. However, continuous expression is required, and this pattern does not reflect the natural expression in the tsetse fly or the influence of other RNA-binding proteins. RBP5 has a single RNA-recognition motif similar to RBP6 and RBP10, whose expression is also upregulated during the life stages in tsetse flies. To evaluate possible contributions to differentiation, we overexpressed RBP5 in procyclic cells alone and in combination with RBP6. Cells overexpressing RBP5-PTP resulted in a moderate loss of fitness, progressive accumulation of cells with 2 nuclei and 2 kinetoplasts (2N2K) and the appearance of multinucleated cells. Conversely, overexpression of non-tagged RBP5 generated a more severe defect, starting immediately following induction. The dramatic increase of 2N2K cells and a greater appearance of multinucleated cells, suggests an irregular cell cycle progression. When developing a dual over-expression system using tetracycline and vanillic acid induction, overexpression of RBP6 did not result in differentiation into any life cycle stage. Together these data suggest that RBP5 might be a regulator of genes involved in the initiation of cytokinesis in T. brucei parasites, however a role in metacyclogenesis cannot be discarded since we were not able to obtain metacyclic parasites.

116 Specific roles for homologous recombination in maintenance of the Trypanosoma brucei subtelomeric VSG (pseudo)gene archive

Krasilnikova, Marijab Crouch, Kathryn; McCulloch, Richard (Wellcome Centre for Integrative Parasitology / University of Glasgow, UK)

African trypanosomes, such as Trypanosoma brucei, are coated with a dense, homogenous variant surface glycoprotein (VSG) layer, encoded by a member of a highly expanded gene family comprising over 2000 genes. In a given cell, one VSG gene is expressed at a time, residing in an active bloodstream expression site (BES). VSG coat switching, largely driven by recombination, occurs throughout an infection as a means of immune evasion, undermining the host immune response and facilitating parasite survival and spread. The VSG gene archive is predominantly located in the silent subtelomeres of large chromosomes and in the minichromosomes, and the machinery involved in the maintenance of this vast gene archive has not been studied in detail. In particular, though a number of DNA repair mechanisms have been documented in T. brucei, whether these have a distinct role in maintaining the VSG repertoire remains unclear. Through the use of recombination-impaired cell lines (rad51-/- and brca2-/- null mutants), we aim to investigate the role of the homologous recombination machinery in VSG archive maintenance and genome stability as a whole. Whole genome sequencing, using both short and long read technology, was used to examine the stability of the VSG archive, BESs and the core genome. Our preliminary results show evidence for extensive gross chromosomal rearrangements within the subtelomeric VSG archive in rad51-/- and brca2-/- cells, but not the core genome or in wild-type cells, suggesting that the HR machinery may have subtelomeric roles in T. brucei that are largely unseen in experiments examining VSG coat switching.

117 SUMO chain mutants promote oscillating parasitemia and extended host survival during T. brucei infection in mice

Iribarren, Paula Ana Coria, Lorena; Di Marzio, Lucía; Berazategui, María Agustina (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina); Saura, Andreu (Instituto de Parasitología y Biomedicina "López-Neyra", CSIC (IPBLN-CSIC), 18016 Granada, Spain); Cassataro, Juliana (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina); Navarro, Miguel (Instituto de Parasitología y Biomedicina "López-Neyra", CSIC (IPBLN-CSIC), 18016 Granada, Spain); Alvarez, Vanina Eder E. (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina, San Martin, Argentina)

SUMOylation is a post-translational modification conserved in eukaryotic organisms involving the covalent attachment of the Small Ubiquitin-like MOdifier (SUMO) to internal lysine residues within target proteins. This modifier usually alters the interaction surface of its substrates, leading to changes in their biological activity, stability or subcellular localization, among other possible outputs. SUMO can be attached as a single moiety or as SUMO polymers in case there are internal acceptor sites in SUMO itself. These chains are important for proteasomal degradation as well as for the formation of subnuclear structures such as the synaptonemal complex in Saccharomyces cerevisiae or promyelocytic leukemia nuclear bodies in mammals.Interestingly, SUMO has been linked to T. brucei antigenic variation, a process where the major surface antigenic protein is replaced by a different variant with certain frequency as a strategy to elude the specific immune response of the host. TbSUMO was found to be enriched in a particular region of the nucleus of bloodstream parasites, colocalizing with the RNA polymerase I at the expression-site body (ESB) within the active variant surface glycoprotein (VSG) expression site. This highly SUMOylated focus (HSF) creates a permissive environment for VSG transcription.In this work, we have examined the role of SUMO chain formation in bloodstream parasites. Using a bacterial strain engineered to produce SUMOylated proteins we confirmed the ability of TbSUMO to form polymers and determined the type of linkage using site-directed mutational analysis. By generating transgenic cell lines unable to form chains, we concluded that SUMO polymerization is not essential for normal growth in vitro. However, using a mouse model of infection remarkable differences between wild-type (WT) and SUMO chain mutant parasites could be observed. While infections with monomorphic parasites caused an unremitting parasitemia and death within 5 to 6 days, chain mutant parasites were able to establish successful infections displaying relapsing and remitting waves of parasitemia and prolonged host survival, resembling the infection pattern observed with pleomorphic parasites. This behavior was not due to differences in VSG expression, as judged by qPCR, FACS and WB, but might be related with the differentiation process as SUMO chain mutants displayed an increased differentiation kinetics from bloodstream to procyclic forms induced by cis-aconitate treatment. Furthermore, mRNA levels of the stumpy markers PAD1 and PAD2 were indeed increased in most SUMO chain mutant samples relative to WT, when parasitemia was at maximum. Taking together these results suggest that parasites with stumpy-like characteristics might be present in mice infected with monomorphic SUMO chain mutants being poli-SUMOylation involved as a negative regulator of parasite differentiation in vivo.

118 Systems-level Analyses to Elucidate Key Purine Stress Response Candidates in Leishmania

Carter, Nicola Yates, Phillip (Oregon Health & Science University); Shepard, Emily; Suchovsky, Skyler (Pacific University School of Pharmacy); Wilmarth, Phillip; David, Larry (Oregon Health & Science University)

Stress response pathways are a key feature of Leishmania biology, enabling survival from nutrient scarcity, as well as parasite adaptation to disparate host environments. Despite being of significance to parasite biology, the molecular events underpinning the parasite's response to changes in the host environment remain largely unknown. To study stress response pathways in L. donovani, we have used purine starvation (a type of nutrient stress) as a model and demonstrated that culture-form promastigotes are able to survive indefinitely without the provision of exogenous purine. Previously, we described a L. donovani cell line, ?gmpr?impdh, in which the survival phenotype from purine scarcity could be manipulated based upon the type of purine restriction imposed. Here, we describe quantitative proteomic and phosphoproteomic analyses to query the molecular response of ?gmpr?impdh cells cultured under normal growth conditions, as well as under three different purine restricted conditions, chosen because they prompt either a survival or nonsurvival phenotype. Across all four treatments, 4735 proteins (59% coverage of the predicted leishmanial proteome) and 12405 phosphopeptides (which mapped to 3039 unique proteins) were identified, highlighting the quality and depth of the dataset. Though a significant number of candidates were observed to be altered in abundance across all three of the purine restricted samples (both survival and nonsurvival conditions), a cohort of 206 proteins and 611 phosphopeptides were altered only in those purine restricted conditions associated with a survival phenotype. These analyses highlight the utility of the ?gmpr?impdh cell line and systems-level approaches for revealing key stress response candidates associated with the survival of purine starvation in Leishmania.

119 TbmtHMG44 is a novel HMG-box protein involved in kDNA maintenance in Trypanosoma brucei

Ochsenreiter, Torsten Hoffmann, Anneliese; Baudouin, Hélène; Amodeo, Simona (University of Bern); Gull, Keith (University of Oxford); Zuber, Benoît (University of Bern); Varga, Vladimir (Institute of Molecular Genetics Prague)

TbmtHMG44 is one of eight HMG-box domain containing proteins in the T. brucei genome.Based on biochemical purifications TbmtHMG44 is interacting with TAC102 a bona fide TAC component of the mitochondrial unilateral filaments. The protein is essential for kDNA maintenance as shown by biochemical and electron microscopy methods. Depletion by RNAi leads to a rapid decrease of minicircles, while maxicircle abundance initially increases. While wild type cells rapidly die upon TbmtHMG44 depletion the “petite” trypanosomes loose kDNA but continue to grow without any defect, indicating the TbmtHMG44 is directly involved in kDNA maintenance. Superresolution microscopy localizes TbmtHMG44 between TAC102 and the kDNA. Future experiments will clarify the role of the HMG-box domain and additional interaction partners of the protein.

120 The Hunt for the Trypanosoma brucei VSG GPI-anchor sn1 Remodelase

Poudyal, Nava Paul, Kimberly (Clemson University)

The surface of Trypanosoma brucei, the causative agent of African Sleeping Sickness, is coated with Variant Surface Glycoproteins (VSGs) that are linked to the cell membrane through glycosylphosphatidylinositol (GPI) anchors. VSG GPI-anchors are initially synthesized with longer fatty acids, but before addition to the C-terminus of the VSG protein, the GPI-anchors are remodeled to replace the two longer fatty acids with two myristates (C14:0). First, remodeling occurs at the sn2 position with deacylation and then addition of myristate via a myristoyltransferase. Second, similar deacylation and myristoyltransferase reactions occur at the sn1 position. Though the sn2 remodelase (aka remodeling myristoyltransferase) has been identified in T. brucei as TbGUP1, the sn1 remodelase is still unknown. Our goal is to identify the VSG GPI-anchor sn1 remodelase. Since GPI remodeling occurs inside the endoplasmic reticulum (ER), we hypothesize the sn1 remodelase is an ER-localized protein with acyltransferase activity. We have screened the T. brucei genome database for candidates using the following criteria: homology to TbGUP1, homology to the other known remodelases, annotated acyltransferase domain homology, ER targeting signals, and transmembrane domains. To date, we have identified 16 candidate genes for the sn1 remodelase. Currently, we are determining the localization of each candidate protein using an epitope-tagging strategy and fluorescence microscopy. For those candidate genes localized to the ER, we will develop RNA interference (RNAi) cell lines to assess their requirement for VSG GPI remodeling using in vitro assays and lipidomic analysis.

121 THE IMPACT OF MIXED SPECIES INFECTIONS ON TRYPANOSOME VIRULENCE AND TRANSMISSION

Venter, Frank Ivens, Alasdair (Centre for Immunity, Infection and Evolution, Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh,); Matthews, Keith R. (Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh E)

Animal African trypanosomes (T. congolense, T. vivax and T. brucei) are clinically important protozoan parasites which are widely distributed across the African continent. These species have a broad host range, infecting wildlife and livestock, and have also been found in mixed infections. These coinfections have been shown to affect both host and parasite, and may select for altered virulence or transmission potential, for example through the cross-recognition of quorum sensing signals. We have explored the consequences of mixed species infection through three approaches. Firstly, we have characterised four T. congolense field isolates, derived from the same geographical region in Zambia, which exhibit different virulence profiles. In each case, virulence correlates of their transcriptome profile have been analysed together with their genomic sequence, with particular focus on conserved genes important in quorum sensing in T. brucei. Secondly, we are coinfecting each of the T. congolense field isolates individually or in combination with T. brucei EATRO 1125 AnTat1.1 in mice, and quantitating the relative prevalence of each species in the coinfection in short term and chronic infections, together with the level of stumpy formation for T. brucei. Finally, we have used a longitudinal survey of trypanosome infected cattle in Kenya (totalling 645 samples) to measure the frequency and dynamics of mixed species coinfections in African livestock over time, this revealing the presence of each of the animal African trypanosome species. Together these studies help further our understanding of the impact of mixed species infections, and their potential to drive changes in the virulence and transmission profiles of parasites in the field.

122 The nuclear export receptors TbMex67-TbMtr2 are required for ribosome biogenesis in Trypanosoma brucei.

Ciganda, Martin Rink, Constance; Williams, Noreen (University at Buffalo)

The nuclear envelope in eukaryotes compartmentalizes nuclear transcriptional synthesis of several classes of RNAs, and provides a physical barrier that separates the biogenesis of ribonucleoproteins (RNPs) from the effector roles these RNPs usually have elsewhere in the cell. In the case of ribosomal RNAs, transport across the nuclear pore is achieved independently for the precursor of the large subunit (LSU) and the precursor of the small subunit (SSU). Exportin 1 (Xpo1), Nmd3, Arx1, and the heterodimer Mex67/Mtr2 interact with these preribosomal particles, mediating translocation through the nuclear pore. In Trypanosoma brucei, both conserved and non-conserved factors facilitate nuclear export of the pre-60S particle. In this work, we examine the role of TbMex67 and TbMtr2 in preribosomal subunit export. Our work shows that specific depletion of TbMtr2 leads to a decrease in steady-state protein levels of TbMex67 and 5S RNP proteins P34/P37 and L5, whereas 5S rRNA levels are unexpectedly not affected. In addition, loss of TbMtr2 leads to an overall decrease in translation and to alterations in the composition of ribosomal subunits. In order to further investigate the connection between these processes in T. brucei, we analyzed levels of rRNA precursors and intermediates throughout the course of depletion of TbMtr2. Loss of TbMtr2 interferes with normal processing of ribosomal RNAs and leads to the accumulation of distinct precursors, especially those of the LSU, i.e. the 5.8kb and 5.0kb precursors of the 28S rRNA and the 0.61kb precursor of the 5.8S rRNA. Overall, our results strongly suggest a close coupling between ribosomal RNA processing and export involving TbMtr2-TbMex67.

123 The RNA-binding proteins RBP7-A and B play distinct roles during the acquisition of infectivity in Trypanosoma brucei

Rojas Sanchez, Saul Tschudi, Christian (Yale School of Publiic Health)

Trypanosoma brucei undergoes a complex developmental program during its life cycle alternating between a mammalian host and an insect vector, thus requiring large changes in gene expression. Our laboratory has established an in vitro metacyclogenesis system which relies on the overexpression of the RNA binding protein 6 (RBP6) in procyclic cells. Here, we began to study the role of RBP7 in this process. RBP7 is present in two almost identical copies in the T. brucei genome: RBP7-A and RBP7-B. Interestingly, while RNAi against the specific 3'-UTR of RBP7-A, in cells expressing RBP6, enhanced the development of metacyclics; overexpression of both RBP6 and RBP7-A inhibited this process. Furthermore, the percentage of metacyclics with 1K1N dropped from ~98% in the parental cell line to ~83% in the RBP7-A RNAi cell line, indicating that metacyclics were no longer arrested in the G1/G0 phase of the life cycle. On the other hand, RBP7-B down regulation, in RBP6 expressing cells, inhibited metacyclic production, but overexpression of both RBP6 and RBP7-B accelerated the appearance of the infective stage. mRNA-seq on the RBP7-A RNAi cell line revealed a large number of differentially expressed transcripts (551) with at least 2-fold change. In this set, 325 and 226 transcripts were up- or down-regulated, respectively. Interestingly, while the group of up-regulated transcripts was enriched in transmembrane transporters; the great majority of down-regulated transcripts encoded mitochondrial proteins. These results showed that, despite being almost 90% identical, RBP7-A and -B play distinct roles during metacyclogenesis.

124 Tidying up the nucleus – which molecular factors build the compartments of VSG gene regulation?

Luzak, Vanessa, Brink, Benedikt; Siegel, Nicolai (LMU Munich)

Evolution – as an elegant architect – has created the organization within a cell in a way that the efficiency of diverse biological processes is maximized. Like an apartment is separated into rooms with different functions such as a kitchen and a bathroom, cellular environments are physically separated by membranes in order to create diverse reaction areas. One such membrane-defined compartment within the cell is the nucleus which harbors the genetic information in form of chromatin. DNA sequences and proteins with various, often opposing function are present in this compartment in a highly condensed manner. In order to prevent chaos and deregulation of gene expression, membrane-less compartments such as the stable nucleolus or transient transcription factories were found to organize the nucleoplasm. I am currently developing an approach called “gradient-seq”, in order to identify proteins, RNA molecules and DNA sequences that create functional compartments in the nucleus of Trypanosoma brucei. Amongst others, molecular factors that establish the regulatory compartments of VSG gene expression can potentially be identified in an unbiased manner by using the “gradient-seq” method.

125 Transcriptomic profiling of cellular and axenic amastigotes of T. cruzi

Smircich, Pablo Bilbao, Lucia (Instituto de Investigaciones Biológicas Clemente Estable / Facultad de Ciencias); Hernández, Fabricio (Facultad de Ciencias); Sotelo-Silveira, José (Instituto de Investigaciones Biológicas Clemente Estable / Facultad de Ciencias); Garat, Beatriz; Pérez-Díaz, Leticia (Facultad de Ciencias)

Trypanosoma cruzi presents a complex life cycle that involves insect and mammalian stages. The epimastigote form that is naturally found in the insect's digestive tract, is widely used as a model stage as it can be easily grown under laboratory conditions. However, many specific and relevant questions related to the biology of the disease involve the use of the intracellular amastigotes. The need to work with parasites from this specific stage has resulted in the development of both axenic (differentiated in vitro from cellular trypomastigotes) and cellular amastigotes models. Even tough the axenic amastigotes are methodologically more convenient, the resemblance of these amastigotes to the in vivo cultured cellular amastigotes has been questioned by the community. In this work we took on a transcriptomic approach to compare the gene expression profiles of amastigotes obtained by both strategies and evaluate the validity of the axenic approach. The data suggest that there are significant differences between both models. Interestingly, cellular amastigotes show more deferentially expressed genes than axenics when compared to cellular trypomastigotes.

126 Trypanosoma brucei TIF3 is a novel telomere protein that is essential for cell viability and affects VSG switching

Schnur, Brittny, Li, Bibo (Cleveland State University, Cleveland, OH)

Trypanosoma brucei causes sleeping sickness in humans and nagana in cattle. While proliferating inside its mammalian host, T. brucei stays in extracellular spaces and elicit strong host immune response. However, T. brucei regularly switches its major surface antigen, VSG, and effectively evades elimination by the host immune system. VSGs are expressed exclusively from subtelomeric expression sites (ESs) in a strictly monoallelic manner. VSG is the last gene in any ES and is located immediately upstream of the telomeric repeats. Telomeres are nucleoprotein complexes at chromosome ends and are essential for genome integrity and chromosome stability. VSG switching can occur through DNA recombination or by changing of transcriptional states of different ESs. We have previously identified TbTRF as the duplex telomere DNA binding factor and both TbRAP1 and TbTIF2 as TbTRF-interacting factors. All these telomere proteins are essential for T. brucei viability. They all suppress DNA recombination-mediated VSG switching, although the underlying mechanisms may vary. Additionally, TbRAP1 is essential for VSG monoallelic expression. Now we have identified another TbTRF-interacting factor in a yeast 2-hybrid screen, which we named TbTIF3 (TbTRF Interacting Factor 3). We found that depletion of TbTIF3 causes an acute cell growth arrest, indicating that TbTIF3 is essential for T. brucei viability. Additionally, a transient depletion of TbTIF3 leads to a small increase in VSG switching frequency, suggesting that TbTIF3 may function in the same pathway as TbTRF and TbTIF2.

127 Trypanosome differentiation

Matthews, Keith R.

Trypanosome

128 TrypSpotting: Identifying Lipid Droplet Proteins in Trypanosoma brucei

Raja, Sripriya

Trypanosoma brucei, the protozoan parasite that causes African sleeping sickness, alternates between mammalian and tsetse fly hosts, which offer dramatically different environments to which the parasite must adapt to survive. In environments where fatty acids are scarce, the parasites rely on fatty acid synthesis and stored fatty acids to meet its needs. A potentially important source of stored fatty acids comes from lipid droplets (LDs), dynamic organelles involved in lipid storage and homeostasis. T. brucei LDs are largely uncharacterized. LD number varies between both parasitic forms, LDs tend to be excluded from the posterior region, and LD number responds to growth in low and high lipid media. To identify proteins involved in T. brucei LD formation and dynamics we screened the TrypTag imaging database for proteins whose GFP-tagged localization resembled the punctate pattern expected for LDs. We identified two candidate genes involved in sterol synthesis (Erg6 and LanS) and three candidate genes annotated as Hypothetical Conserved (Tb927.8.1770, Tb927.11.12280, Tb927.11.9800). We will confirm localization of candidate proteins by epitope-tagging and fluorescence microscopy. To date, localization of myc-tagged Erg6 revealed a distinct but overlapping localization with lipophilic dye LipidTox Red. For each candidate LD gene, we generated RNA interference (RNAi) cell lines for inducible knock-down of expression in procyclic T. brucei. To date, induction of RNAi revealed no effect on growth. Semi-quantitative PCR revealed only a modest knock-down efficiency. We are redesigning RNAi constructs for more efficient knock-down. Candidates showing an LD phenotype upon RNAi induction, and/or confirmed localization to LDs will be further characterized for their role in T. brucei growth and survival.

129 Ubiquitin Conjugating Enzymes in Leishmania: When do they work, with whom, and can they be targeted?

Harris, Daniel

There are few, if any, cellular processes that do not involve ubiquitination in some way. This is likely to be the case in Leishmania, an organism whose dixenous life cycle imposes all manner of profound changes, and whose constitutively expressed genome may be regulated at the protein level. This study aims to identify the ubiquitin conjugating enzymes, the ubiquitinome, and characterise key enzymes for future drug discovery efforts. So far, ubiquitin activating (E1), and ubiquitin conjugating enzymes (E2) expressed in promastigotes have been identified through mass spectrometric methods, as well as a putative ubiquitinome. Additionally, components of the SUMOylation cascade have also been identified. An abundantly expressed E1 is currently undergoing protein crystallography, where its structure will aid in drug design.

130 Variant Surface Glycoprotein Expression in Tissue-resident Trypanosoma brucei

Beaver, Alexander, Zhang, Lucy (Johns Hopkins School of Public Health); Bobb, Bryce; Rijo-Ferreira, Filipa; Figueiredo, Luisa ; Mugnier, Monica (Johns Hopkins School of Public Health)

The extracellular parasite Trypanosoma bruceievades the host immune system by a process of antigenic variation in which the parasite continuously changes its variant surface glycoprotein (VSG) coat to avoid recognition by host antibodies. Previous studies have shown that during an infection parasites occupy the blood and several extravascular spaces, such as the adipose tissue, skin, and lungs. Currently, little is known about VSG expression and diversity in extravascular parasites. Using VSG-Seq to examine VSGs expressed by parasites in the blood and tissues of infected mice, we found that populations of parasites in the tissues, specifically the gonadal fat, expressed a greater diversity of VSGs when compared to parasites in the blood. In addition, the initiating VSG was often still detectable in the tissues after it had been completely cleared from the blood. Our findings suggest that tissue-resident parasite populations experience different environmental pressures leading to independent diversification of their VSGs. This suggests a complex network of semi-independent parasite populations that contribute to antigenic variation during infection. In order to better understand the role of tissue-resident parasites during infection, we are currently conducting experiments exploring the relationship between VSG diversity and antibody-mediated clearance of tissue-resident parasites.

131 VEX1 regulates metacyclic VSG expression in insect stage trypanosomes

Tihon, Eliane Dujeancourt- Henry, Annick; Glover, Lucy (Institut Pasteur)

Trypanosomes undergo several developmental transitions during their life cycle. Critical to transmission from the insect vector to the mammalian host is differentiation into metacyclic forms in the tsetse fly salivary glands, through a process known as metacyclogenesis. Metacyclic trypanosomes acquire mammalian infectivity by expressing metacyclic variant surface glycoprotein (mVSG) on their surface, but the mechanisms underlying their activation are unknown. The recently described protein VEX1 (Glover et al, PNAS, 2016) regulates the expression of both variant surface glycoprotein(VSG) and mVSGs in bloodstream form parasites and overexpression of RNA binding protein 6 (RBP6) in procyclic trypanosomes induces metacyclogenesis in vitro. Conditional knock-down and overexpression of VEX1 in established insect stage parasites resulted in derepression of mVSG genes only. Following metacyclogenesis VEX1 relocalised to form a single focus in metacyclic cells, in vitro and in vivo. Using a doubly inducible system that allows us to temporally control the induction and knock down of both RBP6 and VEX1 we established a system to assess the role of VEX1 in metacyclogenesis and mVSG expression.

132 VSG length dependent growth rate in Trypanosoma brucei

Scheidt, Viktor Horn, David

Bloodstream African trypanosomes express super-abundant variant surface glycoproteins (VSGs), which form a dense coat on each cell; approximately 10% of total cell protein. To avoid detection by the host's immune system, the cells undergo antigenic variation by switching their VSG; using a repertoire of thousands of different VSG genes and pseudo-genes. Importantly, only one is expressed at the time. The active VSG is transcribed in one of about 20 subtelomeric expression sites (ESs) and switching is triggered by a DNA double-strand break that results in homologous recombination and replacement of the active VSG with a new VSG, most often from another ES (VSG length range in ESs is 466-537 aa). Recently, mathematical modelling using in vivo data (PMID:23853603; PMID:25814582) showed that T. brucei parasites expressing shorter VSGs appear in early infection and parasites with longer VSGs appear later. This suggested that cells with shorter VSGs grow faster than cells with longer VSGs (PMID:30026531). To test this hypothesis we are assessing VSG length dependent growth rate of T. bruceiin vitro. I created a double strand break upstream of the active, short VSG-2 gene (476 aa), using inducible CRISPR/Cas9. This approach yielded millions of switched cells, which were subsequently outgrown by the VSG-2 expressing population. Currently, several clonal populations expressing VSGs of different length are being assessed; with preliminary data supporting our hypothesis. Thus, length-dependent growth can explain the prevalence of short VSGs in vitro and the temporal hierarchy of VSG prevalence observed in vivo and would allow a cohort of VSGs to support a more persistent infection. Given VSG abundance, differences in metabolic load may explain differential growth.

133 What happens inside the nucleus when trypanosomes switch expression of VSGs?

Rabuffo, Claudia

African trypanosomes are eukaryotic parasites that live in the bloodstream of their mammalian hosts. To evade the host immune response, individual trypanosomes periodically change the expression of their major surface antigens, the variant surface glycoproteins (VSGs). Our aim is to visualize the switch of VSG expression and to study its effects on chromatin accessibility and genome organization. To this end, we want to identify cells during VSG switching and just after it. To isolate a sufficiently large number of such cells, we will take advantage of a novel machine-intelligence technology referred to as intelligent Image-Activated Cell Sorting (IACS). IACS integrates fluorescence microscopy and sorting of cells in real-time. Thus, IACS should allow us to image switchers and to isolate them for downstream analyses such as scRNA-seq, ATAC-seq or Hi-C that will provide information regarding the transcriptome, DNA accessibility and genome organization.

134 Whole genome RNAi library screens identify repressors of metacyclic VSG expression site transcription in bloodstream form T. brucei

Davies, Carys Sioutas, George; Sidhu, Haneesh; Hall, Belinda (Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, London); Wickstead, Bill (School of Life Sciences, University of Nottingham, UK); Alsford, Sam (London School of Hygiene and Tropical Medicine, London); Rudenko, Gloria (Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, London)

Trypanosoma brucei relies on antigenic variation to survive extracellularly in the bloodstream of its mammalian hosts. The strict mono-allelic expression of the protective Variant Surface Glycoprotein (VSG) from a single expression site (ES) is crucial to this process. There are two types of ES that VSG can be expressed from; bloodstream form ESs (BESs), and metacyclic form ESs (MESs). Both of these must be strictly regulated in bloodstream form (BSF) trypanosomes for antigenic variation to be effective.MESs are substantially shorter in length than BESs, contain fewer repetitive sequences, and are monocistronic transcription units. It is unknown whether different mechanisms of repression are required to regulate these different types of ESs. To identify factors involved specifically in the down-regulation of MESs in BSF trypanosomes, an RNAi library screen was carried out followed by high throughput RITseq analysis. We identified two genes that play a role in MES repression in BSF trypanosomes: SAP, and Elf1.SAP contains a predicted SAP DNA binding domain. SAP domains are typically found on proteins associated with chromatin and have been implicated in the organisation of chromatin domains. SAP is localised in the nucleus, with particular enrichment at the nuclear periphery. Elf1 is a transcription elongation factor homolog that also localises in the nucleus with an enriched focus that appears to be associated with the active ES. RNAi mediated knockdown of Elf1 or SAP combined with RNAseq revealed de-repression of both MESs and BESs, but different effects proximal or distal to promoters. Further characterisation of these candidate genes will provide more insight into the transcriptional regulation occurring at MESs, and potentially all telomeric VSG expression sites.

135 ZC3H20 and ZC3H21 in Trypanosoma brucei: the targets and functions.

Liu, Bin Marucha, Kevin; Bajak, Kathrin; Nascimento, Larissa; Clayton, Christine (Zentrum für Molekulare Biologie der Universität Heidelberg)

In Trypanosoma brucei, RNA-binding proteins are critical for in controlling mRNA processing, turnover and translation. The parasite proliferates as long slender forms in the bloodstream of the mammalian host, and differentiates at high density into nondividing stumpy forms which are infectious to Tsetse flies. Within the tsetse midgut they differentiate into procyclic forms. We previously showed that the RRM-domain protein RBP10, which is expressed exclusively in the bloodstream form, targets procyclic-spcific mRNAs for degradation.Among the mRNA targets of RBP10 are those that encode three proteins that have two CCCH zinc finger domains: ZC3H20, ZC3H21, and ZC3H22. Correspondingly, all three proteins and mRNAs are more abundant in procyclic than bloodstream form trypanosomes. ZC3H20 and ZC3H21 are similar, but not identical, in the CCCH RNA-binding region, while ZC3H22 is more distantly related. ZC3H20 and 21 have, at their C-termini, a motif that interacts with MKT1. Tethering results showed that ZC3H20 and ZC3H21 increase expression of a reporter mRNA, and that the MKT interaction motif of ZC3H20 is essential for this activity. This suggests that ZC3H20 acts via the MKT-PBP-XAC1-LSM12-PABP complex.ZC3H20 is detectable in stumpy forms, whereas ZC3H21 appears only upon procyclic differentiation. Correspondingly, Cells lacking ZC3H20 could not make stumpy forms, and were unable to form procyclic forms in vitro. The defect could be complemented by exogenous ZC3H20, but not ZC3H21, suggesting differing activities. In contrast, RNAi targeting ZC3H20 or ZC3H21 individually caused only a very slight or no defect in established procyclic forms, but RNAi targeting both of them suppressed growth. Results of a previous study suggested that ZC3H20 binds to, and stabilizes, two mRNAs encoding procyclic-specific membrane proteins. Our new RNA-pull-down results for ZC3H20 and ZC3H21, combined with transcriptomes of the depleted cells, suggested that the proteins have overlapping, but distinct profiles. Both preferentially bind to, and stabilize, mRNAs encoding plasma membrane and mitochondrial proteins, but each has additional, more specific targets. Loss of both proteins in procyclic forms affects many mRNAs that are not bound by either protein, resulting in a transcriptome switch towards a bloodstream-form pattern. The mechanism by which the switch is induced is not obvious, but one possibility is that it is caused by impaired energy metabolism.

136 Identification and function of the VSG transcript-bound proteome: methodological development and preliminary findings

ERBEN, Esteban D. *STROHMENGER, Soren (DKFZ); CLAYTON, Christine (ZMBH); PAPAVASILIOU, Nina (DKFZ)*

The selection of mRNAs that bind to individual RNA-binding proteins is routine, but reliable methods to select proteins that are bound to individual mRNAs are as yet not available. To address this problem, we have chosen bloodstream-form trypanosomes, because at least 5% of their total mRNA encodes the Variant Surface Glycoprotein (VSG), and it is all produced from a single gene. A conserved 3'-terminal region of the VSG transcript regulates both mRNA levels and translation. As control we use the mRNA encoding tubulin, which comprises about 1% of the total mRNA. We have succeeded in enriching the VSG mRNP >500-fold using transcript-specific pull-down with biotinylated DNA oligonucleotides. Preliminary mass spectrometry results suggest that some components of the VSG and tubulin-encoding mRNPs are specific.As a complementary approach, we have implemented a screen based on dual-color fluorescence reporter construct in which the open reading frames that encode eGFP and mCherry are linked to the actin and VSG 3'-UTRs, respectively. The reporter cell line was transfected with an inducible overexpression library. Cells exhibiting a relative increase or decrease in normalized mCherry expression after induction were sorted; the over-expressed genes that each contain are now being characterized.Finally, we assembled cell lines expressing boxB aptamer-tagged VSG and tubulin mRNAs which will allow for mRNA-protein complex purification using the high-affinity lambda-SBP tag and for protein detection via proximity-dependent biotinylation. We expect these complementary approaches to contribute to a better understanding of the processes governing the complex life of mRNA with special focus on the VSG transcript. Preliminary results for some candidate mRNP-specific proteins will be reported.

137 Identification of Biomarkers of Trypanosoma cruzi Infected Cells

Acharyya, Nirmallya Serna, Carylinda (National Cancer Institute, NIH); Silberstein, Erica; Acosta, David; Nagarkatti, Rana; Debrabant, Alain (Food and Drug Administration)

Chagas disease is caused by the blood-borne parasite Trypanosoma cruzi. It is endemic in Latin America and impacts other countries due to population migration. One third of infected individuals will develop severe cardiac or gastrointestinal symptoms. T. cruzi parasites infect and grow within nucleated cells in various tissues. The goal of this study is to determine whether parasite proteins are expressed at the surface of infected host cells. Using a proteomics approach, we identified seven candidate parasite proteins expressed at the surface of infected MK2 cells in vitro. Three of these proteins, HSP70, GRP78 and Mucin, which showed low sequence homology with their host homologs, were recombinantly expressed in bacteria and polyclonal antibodies made in rabbits. The anti-HSP70, anti-GRP78 and anti-Mucin antibodies showed parasite specific single band reactivity by Western blot and high antibody titers by ELISA, with T. cruzi trypomastigote and epimastigote cell extracts. These antibodies also reacted with parasites by IFA using fixed and permeabilized preparations of both extracellular parasites and T. cruzi infected MK2 cells. Of significance, IFA performed with live, non-fixed, non-permeabilized cells showed labeling of the infected host cell surface membrane. These “live IFA” results are consistent with our proteomics data and suggest the parasite HSP70, GRP-78 and Mucin proteins are present at the surface of infected MK2 cells in vitro. These proteins represent ideal targets for probes (e.g. antibodies or aptamers) used in imaging and drug targeting studies to either identify new sites of parasite persistence in the host or to improve treatment of Chagas disease by targeted drug delivery.

138 Identification of kinetoplastid parasite glucose uptake inhibitors

Call, Daniel Vance, Jacob (Brigham Young University); Voyton, Charles (Clemson University); Choi, Jongsu (Brigham Young University); Werbovetz, Karl (The Ohio State University); Golden, Jennifer (University of Wisconsin-Madison); Morris, James (Clemson University); Christensen, Kenneth (Brigham Young University)

The bloodstream form (BF) of Trypanosoma brucei is the infectious agent responsible for African trypanosomiasis. Since BF T. brucei relies solely on glycolysis for ATP production, glucose uptake and metabolism is an attractive target pathway to treat T. brucei infection. To identify small molecules that inhibit glucose uptake and can be potential therapeutics, a 25,000 chemical library was screened for molecules that alter intracellular glucose in T. brucei. We used novel genetically-encoded FRET glucose sensors to monitor the impact of individual compounds on cytosolic and glycosomal glucose concentrations. Potential hits were validated for glucose uptake via their impact on uptake of the fluorescent glucose analog, 2-NBDG, and were assessed for growth inhibition in T. brucei and Leishmania donovani, a related kinetoplastid parasite. Human cell toxicity and impact on glucose uptake were assessed in GLUT1 expressing HEK293T cells. Two distinct chemotypes were identified and their structural-activity relationships (SAR) were evaluated. BDGR-9054 (first chemotype) has a 2-NBDG uptake inhibition EC50 of 0.84 µM in T. brucei procyclic form (PF) and growth inhibition EC50 of 8.5 µM in BF. Also, a growth inhibition EC50 of 8.2 µM in Leishmania donovani (axenic amastigotes) and minimal HEK293T cell toxicity (EC50 21.8 µM) was observed. BDGR-9054 is racemic; hence, the active enantiomer is expected to have at least a 2-fold potency increase relative to measured values. BDGR-9032 (second chemotype) has glucose uptake inhibition EC50 of 0.28 µM (PF) and growth inhibition EC50 of 4.0 µM (BF), with negligible HEK cell toxicity (EC50 > 50 µM). We have identified novel antikinetoplast compounds and performed preliminary SAR; two compounds are ready for further optimization.

139 Impact on virulence of flagellar pocket protein kinases in Leishmania mexicana

Neish, Rachel Mottram, Jeremy (University of York)

Leishmania belongs to a group of unicellular parasites which are highly polarised and depend on cytoskeletal remodelling for virulence and differentiation to its different life-cycle stages. They have a single anteriorly positioned flagellum, central to which the flagellar pocket is the exclusive site for exo/endocytosis and attachment of the flagellum. Furthermore, assembly of the flagellum and flagellar pocket proteins are integral to cytokinesis and cell morphology. There have been many studies on the flagellum and associated organelles in trypanosomatids, nevertheless regulatory pathways of phosphorylation involved in flagellar remodelling in differentiation are poorly understood.

We applied the CRISPR-Cas9 genome editing system to generate a library of 154 protein kinase knockout mutants in L. mexicana to identify pathways involved in differentiation and virulence. This library was used in high throughput competition assays using in vitro (macrophage infection) and in vivo (murine model) assays. Introduction of a unique barcode allowed us to identify loss of fitness during the infection via DNA sequencing. A few selected knockout cell lines and their corresponding episomal add-backs were used to validate the screen.

We also generated mNeonGreen endogenously tagged cell lines in L. mexicana for localisation of all the protein kinases. From these screens, a total of seven protein kinases localised to the flagellar pocket. Four of which had a reduction in fitness in mice, of those, three localised solely to the flagellar pocket region and the other also localised to microtubule quartet. This kinase is highly conserved among the trypanosomatids, with 62% to T. brucei. These studies provide new insights into regulation of flagellar pocket function via phosphorylation.

140 In Trypanosoma brucei, activity of fructose, 1-6, bisphosphatase, an enzyme involved in gluconeogenesis, is upregulated in high-glucose conditions

Wilkinson, Christina Morris, Meredith (Clemson University)

Trypanosoma brucei inhabits multiple environments that differ in their nutrient availabilities. In high-glucose environments such as the mammalian bloodstream, parasites generate ATP via glycolysis. In low-glucose environments parasites must generate ATP via alternative metabolic pathways such as gluconeogenesis (GNG), which generates glucose from non-carbohydrate carbon sources. GNG has been demonstrated in insect stage, procyclic form (PF), parasites (Allmann et al., 2013) and a key enzyme in this pathway, fructose-1,6-bisphosphatase (FBPase), is essential for parasite development in the fly (Wargnies et al., 2018). Recent studies also indicate that bloodstream (BF) parasites utilize GNG (Kovarova et al., 2018). Using antibodies against recombinant protein, we found that FBPase expression fluctuates with life-cycle stage and extracellular glucose levels. PF cells grown in a high-glucose media have almost twice the amount of FBPase per cell as PF grown in low-glucose media and BF parasites express 50% less FBPase as PF in high glucose. It was surprising to us that FBP expression levels were higher under conditions in which GNG is expected to be less active. While T. brucei clearly uses GNG, and western analyses indicate that FBPase is expressed in both PF and BF parasites, FBPase activity has been difficult to detect in cell lysates. Using a modified and more sensitive FBPase assay, we have detected low levels of FBPase activity in PF parasites that is reduced in FBPase RNA-interference cell lines. While FBPase protein levels were highest in PF parasites, FBPase activity was 2-fold higher in BF parasites. These results suggest that GNG flux is not directly correlated with FBPase activity and that the enzyme may play alternative functions in parasite metabolism.

141 Inducible suicide system in Leishmania mexicana

Podesvova, Lucie Yurchenko, Vyacheslav (University of Ostrava, Faculty of Science, Life Science Research Centre, Czech Republic)

Leishmaniases are closely related diseases caused by the flagellate protozoan Leishmania spp., representing a global health problem with over 350 million people at risk and an annual incidence rate of 2–10 million worldwide. The diseases are affecting human and animal populations mainly in the tropical and subtropical regions with the clinical manifestations varying from spontaneously healing skin lesions to progressive and possibly fatal visceral infections. Current treatment is associated with negative side effects, high price and increasing parasite drug resistance. That is why the scientific community has spent a considerable effort to develop an effective vaccination approach.Here, we present an inducible suicide system in Leishmaniamexicana, a causative parasite of cutaneous leishmaniasis. The system is based on the inducible protein stabilization system that was recently established in this parasite (1). Destabilization domain derived from Escherichiacoli dihydrofolate reductase (ecDHFR) destabilizes the fused protein and, thus, induces its degradation by proteasomes. This process is reversible upon addition of the specific ligand, Trimethoprim. We have tested the domain destabilization properties in combination with several toxic proteins and/or antimicrobial peptides. One such toxin, BnSP-7, a basic phospholipase A2 from Bothropspauloensis snake venom, caused significant division delay and cell death of the parasite upon addition of the stabilizing ligand. We believe that such inducible suicide system and resultant live attenuated parasites could be used as a potential vaccination strategy against Leishmaniamexicana.1 Podešvová, L., Huang, H., Yurchenko, V. Inducible protein stabilization system in Leishmaniamexicana. Mol. Biochem. Parasitol., 2017, 214: 62-64.

142 Inhibitors of CLK1 protein kinase in kinetoplastids

Bower-Lepts, Christopher Saldivia, Manuel; Mottram, Jeremy (University of York); Rao, Srinivasa (Novartis Institute of Tropical Disease)

Protein kinases are attractive targets in drug discovery because they regulate essential cellular processes ranging from growth to differentiation and are amenable for inhibition by small molecule compounds. Protozoan protein kinases differ markedly from their mammalian counterparts making them promising targets for selective drug inhibition. A focused high-throughput screen of a Novartis kinase-biased inhibitor library identified the amidobenzimidazole (AB) compound series as scaffolds with high potency against Trypanosoma brucei growth in vitro in addition to favourable drug-like properties and selectivity over mammalian cells. We identified CLK1 as the in vivo target; CLK1 (KKT10) is a CMGC-family protein kinase and constituent of the divergent kinetochore structure in these parasites. We expressed and purified recombinant T. brucei and L. mexicana CLK1 and developed a protein kinase assay for assessing the inhibitory activity of the AB compounds, leading to a structure activity relationship. In vitro recombinant protein assays demonstrate that T. brucei CLK1 phosphorylates KKT2, another protein kinase in the kinetochore, at a conserved serine residue and mutation of this residue disrupts this phosphorylation event. AB compounds were also found to have potent activity against Leishmania. This work therefore describes a novel drug target for kinetoplastid parasites and a promising chemical probe for dissecting the function and structure of the divergent kinetochore in these parasites.

143 Intrinsic susceptibility to Amphotericin B in clinical isolates of Leishmania subgenus Viannia.

Echeverry, Maria Franco, Carlos (Centro Dermatológico Federico Lleras Acosta); Ovalle-Bracho, Clemencia (CentroDermatológico Federico Lleras Acosta); Saavedra, Carlos (Facultad de Medicina, Universidad Nacional de Colombia)

Introduction: Amphotericin B (AmB) has gained value for the treatment of all forms of leishmaniasis. However therapeutic failure when using AmB has begun to be reported. In order to assess what is driving AmB susceptibility in field isolates associated to Cutaneous Leishmaniasis (CL), this study was aimed to determine the In vitro susceptibility to AmB of 100 Leishmania spp isolates from Colombian patients suffering from CL. Methods: Parasite species was determined by PCR-RFLP; intracellular amastigotes were produced for each isolate by infecting U937-derived macrophages. 24 hours-infected cells were exposed to different AmB concentrations during 96 hours. Parasite load was quantified by qPCR and AmB half maximal effective concentration (EC50) was determined by using the PROBIT model. The isolates were classified according to their EC50 through Ward's hierarchical method. When available, information regarding Miltefosine and Antimony susceptibility was analysed for each isolate. Results: The typified species distribution was: L. (V.) panamensis 54%, L. (V.) braziliensis 30% and L. (V.) guyanensis 16%. AmB median EC50 for L. (V.) panamensis was 0.2515 µM (CI: 0.0012-9,3 µM), for L. (V.) braziliensis 0.4173 µM (CI: 0.003-8.73 µM) and for L. (V.) guyanensis 0.1475 µM (CI: 0.003-1.018 µM). AmB variation in susceptibility was found between species. L. braziliensis isolates were more frequently associated with low AmB-susceptibility ($p<0.00001$). Despite the fact that a high number of isolates simultaneously decreased in susceptibility to AmB and Miltefosine, there was no statistically significant “cross-resistance” between the two treatments. Conclusion: AmB therapeutic failure observed in CL could be associated with intrinsic parasite low susceptibility to AmB.

144 Investigating ciliopathy-linked polymorphisms in IFT172 using precision Cas9 mediated genome editing in Trypanosoma brucei

Hutchinson, Sebastian Jung, Jamin; Bastin, Philippe (Trypanosome Cell Biology Unit, Institut Pasteur & INSERM U1201, 25-28 Rue du Dr Roux, Paris, 75015 France.)

The trypanosome flagellum is an essential organelle, as it is required for progression through the tsetse fly and infection of the mammalian host. In addition, the trypanosome flagellum is an excellent model system for the study of flagellar construction and maintenance, as both of these processes can be studied in a single cell. Intraflagellar transport is a process that carries flagellar building blocks along microtubules to the sight of construction at the flagellar tip. In trypanosomes, intraflagellar transport (IFT) genes, such as IFT172 (Tb927.10.1170) are required for flagellar construction. IFT proteins are well conserved across all ciliated organisms (cilium and flagellum are interchangeable terms) for example 70 % of residues are conserved between human and T. brucei IFT172 homologs. A range of diseases in humans termed ciliopathies are linked to mutations in these IFT genes. We are using CIRSPR/Cas9 mediated precision genome editing of TbIFT172 to explore the role of three human ciliopathy associated mutations in trypanosome IFT172 function (V412R, D469 and R470 deletion, C1737R) and flagellar construction. We have assembled procyclic form strains based on the genome editing system published by Rico et al 2018. The Cas9 gene is stably integrated into the genome and its expression is tetracycline-inducible whereas constitutive guide expression constructs are integrated into a ribosomal spacer targeting each of the three IFT172 regions. Additionally, a single allele of IFT172 is 5' tagged in its native context with mNeonGreen as a reporter of IFT172 function. We initially demonstrated normal trafficking of IFT172 in the flagellum by video microscopy. Next, we established Cas9 mediated disruption of IFT172 following induction of protein expression. For precise genome editing, we induced Cas9 expression in these strains, and after 24h nucleofected 100 b ssDNA repair templates. These populations were then subcloned and screened by a qPCR assay involving no DNA extraction steps. We have also performed genome sequencing on nucleofected populations. We will next characterize the effect of these mutations on flagellum construction through IFT particle construction, flagellar gating and motility.Rico E, Kovárová J, Jeacock L and Horn D. (2018) Inducible high-efficiency CRISPR-Cas9-targeted gene editing and precision base editing in African trypanosomes. *Scientific Reports. 8:7960.

145 Investigating Dynamics in the Kinetoplastid Mitochondrial Network

DiMaio, John Malfara, Madeline; Povelones, Megan (Penn State Brandywine)

Kinetoplastids have a single mitochondrion per cell. In most species, the mitochondrion is a branched network, with the mitochondrial DNA (kDNA) located at the extreme end. This configuration may present challenges for maintaining the health of the organelle and partitioning during cell division. We are using Crithidia fasciculata as a model kinetoplastid to study the properties of kinetoplastid mitochondrial networks. The cell cycle of C. fasciculata resembles that of Leishmania species, in that cells grow shorter and wider as the cell cycle progresses. Using a C. fasciculata cell line expressing mitochondrial GFP, we have found that mitochondrial growth occurs primarily in G1. Mitochondrial division is coincident with cytokinesis. One of the advantages of C. fasciculata is that an immotile, haptomonad form, which allows the parasites to attach to the hindgut of their mosquito host, can also be generated in vitro. This form is perfectly suited for time-lapse confocal imaging of live cells. Using this approach, we can observe mitochondrial remodeling events throughout the C. fasciculata cell cycle in both adherent haptomonads and immobilized nectomonads. These events include tubule fission, fusion, sliding, budding, and the occasional formation of fenestrated sheets. The type and frequency of these events resembles mitochondrial dynamics in yeast and mammalian systems. Kinetoplastids have some predicted homologs of canonical mitochondrial division and fusion proteins, including a dynamin-like protein and a mitofusin-like protein. While DLP has been implicated in mitochondrial division in Trypanosoma brucei, the precise role of these proteins in maintenance or remodeling of mitochondrial structure has not been described. We are using genetic approaches such as CRISPR to investigate the role of candidate proteins in mitochondrial dynamics.

146 Investigating the Role of Potassium Ion Flow in Trypanolysis

Oprea, Yasmine Ko, Daphne (CUNY Hunter College); Verdi, Joey (CUNY Graduate Center); Raper, Jayne (CUNY Hunter College)

African trypanosomes are extracellular parasites that cause African trypanosomiasis, or sleeping sickness. They are transmitted by infected tsetse flies to mammalian hosts, but humans and certain primates are able to resist infection by Trypanosoma brucei brucei because of innate immune factors known as trypanosome lytic factors (TLFs). TLFs are high-density lipoproteins that contain the trypanolytic protein Apolipoprotein L-1 (APOL1). APOL1 forms cation channels in the plasma membrane of the trypanosome, causing ion flux across the plasma membrane. After channel formation in the membrane, there is an initial influx of sodium ions, followed by chloride influx and a loss of osmoregulation resulting from uncontrolled ion flux. We hypothesize that the initial sodium influx is counterbalanced by potassium ion efflux through potassium leak channels. Trypanosomes have a genetically encoded heterodimeric plasma membrane potassium channel that is essential to survival, and we hypothesize that blocking this channel would accelerate lysis. To investigate this, we used the potassium channel blocker barium chloride to block the transport of potassium ions across the plasma membrane and performed TLF-mediated trypanolysis assays using TLF-containing high-density lipoprotein isolated from human plasma. We observed that TLF kills parasites more rapidly in the presence of barium, although barium itself does not kill the parasites. However, barium is not entirely specific for potassium channels. Thus, we would like to confirm these biochemical experiments byusing reverse genetics via targeted knockdown of the potassium channel in the presence of TLF and repeating these trypanolysis assays. This would help us better understand the mechanism by which APOL1-mediated trypanolysis occurs.

147 Investigation of iron superoxide dismutase role in Leishmania infantum

Santi, Ana Maria Silva, Paula; Diefenbach, Katharina (René Rachou Institute, FIOCRUZ MINAS); Beverley, Stephen (Washington University School of Medicine in St. Louis); Murta, Silvane (René Rachou Institute, FIOCRUZ MINAS)

Leishmaniasis is a neglected disease caused by protozoans of the genus Leishmania. The antioxidant defense in those parasites differs from that into the host, which could be exploited as a potential target for therapy. Iron superoxide dismutase A (FeSODA) is a metalloenzyme involved in the antioxidant defense by converting superoxide radicals to oxygen and hydrogen peroxide inside the mitochondria. Although the fact that SOD is also found in humans, the catalytic site of human's superoxides are different, containing Cu/Zn and Mn instead the Fe. To investigate the role of FeSODA in Leishmania infantum, we attempt to generate FeSodA null mutants by different methods: by two rounds of conventional homologous gene replacement and also by CRISPR/Cas9. For CRISPR/Cas9, we tested two different approaches. First, we transfected L. infantum with a plasmid for SpCas9 and sgRNA expression, and also with a donor DNA containing stop codons in different frames to disrupt FeSodA sequence. In the second approach, L. infantum stably expressing SpCas9 and T7 RNA polymerase were transfected with the DNA templates for the gRNAs, which are in vivo transcribed by the T7 RNA polymerase; and also with a donor DNA for the replacement of FeSodA sequence by a selectable marker. In all attempts, although we were able to replace or disrupt both alleles of the gene, a third copy was retained via aneuploidy. Western blot analysis of the FeSodA-/-/+ parasites obtained by two rounds of conventional homologous gene replacement, revealed that the FeSODA expression is reduced. Phenotypic analysis showed that those parasites were more susceptible to oxidative stress generated by menadione in vitro and they were less able to maintain the infection in THP-1 macrophages when compared to wild-type. We are currently evaluating the FeSodA-/-/+ amastigotes for their susceptibility to trivalent antimony.Financial Support: FAPEMIG; CNPq; CAPES; UGA/FAPEMIG; Projeto INOVA Fiocruz

148 KHARON interacting proteins KHAP1 and KHAP2 are essential for growth of Leishmania amastigotes

Kelly, Felice D. Hatfield, Jess (University of Notre Dame); Landfear, Scott (Oregon Health Sciences University)

KHARON is a multifunctional protein that is conserved in all kinetoplastid parasites whose genomes have been sequenced. KHARON was discovered in Leishmania mexicana as a protein that interacts with the flagellar targeting domain of the glucose transporter GT1 and is required for GT1 trafficking to the flagellum. It is also essential for amastigote viability inside macrophages, as ?kharon knockout amastigotes fail to undergo cytokinesis and die. KHARON localizes to the subpellicular microtubules, the mitotic spindle, and the base of the flagellum, exhibiting multiple locations and probably multiple functions. BioID and TAP tagging suggest that KHARON is a member of at least one multi-protein complex.To separate the multiple functions of KHARON we are looking for partner proteins with more restricted localizations that mediate specific functions of the KHARON complex. We have identified two KHARON-associated proteins, KHAP1 and KHAP2. KHAP1 and KHAP2 were initially identified by proximity-based biotin labeling, and later detected in unbiased mass-spectrometry pull-down experiments. Both proteins localized to the sub-pellicular microtubules, but their localizations to other microtubular structures differed: KHAP1, but not KHAP2, localized to the mitotic spindle and KHAP2, but not KHAP1, localized to the base of the flagellum. Deleting either KHAP1 or KHAP2 impaired amastigote growth, but neither altered protein trafficking to the flagellum. Deleting KHAP2 also resulted in slower growth rates in promastigotes. Experiments are ongoing to further characterize the functions of these proteins and to determine how KHARON mediates targeting of membrane proteins to the flagellum and why it is required for cytokinesis. Parallel experiments are ongoing with KHARON from Trypanosoma brucei, where it plays similar roles to those defined in L. mexicana.

149 Lysine acetylation: the rise of the rival, a perspective from Trypanosomatids

Moretti, Nilmar Barbosa Leite, Ariely; Maria dos Santos Moura, Leila; Caroline de Castro Nascimento, Ana; Maran, Suellen; Schenkman, Sergio (Federal University of Sao Paulo)

Like protein phosphorylation, the posttranslational addition of acetyl groups to lysine residues of eukaryotic and prokaryotic proteins has been known for decades. However, while in the beginning the main focus of the works was to understand the function of histone acetylation in the regulation of chromatin structure and gene expression, in the last years hundreds of proteins with acetylated lysine have indeed been identified. These non-histone acetylated proteins are from different cellular compartments and involved in a variety of biological processes, such as, metabolism, translation and oxidative stress response. Recently, we described the Trypanosoma cruzi epimastigotes and Trypanosoma brucei procyclic and bloodstream forms acetylomes, and found very distinct acetylation profiles between them. Notably, only 27 proteins were common among T. brucei forms, reflecting differences in the mechanisms of adaptation of parasite during its life cycle. Interesting, the differences in the acetylation was more evident in the glycolytic enzymes, where lower levels were detected in bloodstream forms, which use glycolysis as main energy source, compared to procyclic, which relies on oxidative phosphorylation for ATP production. Biochemical assays demonstrated that fructose 1,6-bisphosphate aldolase is regulated by acetylation, suggesting a new mechanism to regulate glycolysis in this parasite. On the other hand, T. cruzi acetylome showed the presence of several enzymes involved in oxidative stress response acetylated. Among these enzymes, we detected the superoxide dismutase A that is negatively regulated by acetylation as demonstrated in humans. Acetylated human superoxide dismutase is activated when deacetylated by the sirtuin SIRT3, a NAD+-dependent lysine deacetylase. Using biochemical assays we were able to demonstrated that acetylation can also negatively regulates parasite enzyme activity. Also, using parasites overexpressing T. cruzi SIRT3 we observed an increased in resistance under oxidant agents compared to wild-type parasites. Finally, we are now studying the function of protein acetylation in Leishmania parasites. In conclusion, our data demonstrated that acetylation play an important role in regulating essential biological processes in Trypanosomatids.Funding: FAPESP and CNPq

150 Natural IgM antibodies bind to Trypanosome Lytic Factor 1 to form TLF2

Savran, Michelle Verdi, Joey; Raper, Jayne (Hunter College CUNY)

Trypanosome lytic factors 1 and 2 (TLF) are serum-soluble high-density lipoprotein complexes that mediate human immunity by delivering the channel-forming protein apolipoprotein 1 (APOL1) to African trypanosomes. TLF2 is distinguished from TLF1 by the addition of non-covalently associated immunoglobulin M (IgM) antibodies. TLF2 is present in all human serum regardless of infection history, but at a lower quantity than TLF1. Based on this, we hypothesized that infection leads to an increase in IgM available to bind to TLF1 and therefore an increase in TLF2. We first assessed this hypothesis by measuring TLF1 and TLF2 in human serum and observed a shift in equilibrium from TLF1 to TLF2 after infection in sleeping sickness patients. We recapitulated the shift in equilibrium in an animal model using mice expressing APOL1 and Hpr after hydrodynamic gene delivery (HGD) and observed that while mice make only TLF1 initially, TLF2 levels rise after trypanosome infection. The increase in TLF2 levels was correlated with the rise of natural IgM antibody levels in serum in both humans and animal models, which suggested that the TLF2 associated IgM was a trypanosome elicited immune response. However, since all human serum samples contain TLF2 (TLF1-IgM) regardless of infection history, we hypothesized that these IgMs could be expanded polyclonal non-specific natural IgMs. We investigated the origin of the antibody by challenging mice without activation induced cytidine deaminase (AID), an enzyme that facilitates class-switch recombination and somatic hypermutation of antibodies, resulting in mice that can only produce natural IgM antibody. We found that AID-/- mice clear parasite infection at similar rates to wild-type mice, which have the capacity to mature the antibody response. These data indicate that natural IgM antibodies play a primary role in the immune response to trypanosomes and suggest that the TLF2-associated antibodies are indeed natural IgMs.

151 On the way to the T. brucei Flagellar Pocket Collar

Bonhivers, Melanie Isch, Charlotte; Landrein, Nicolas (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France); Majneri, Paul; Pivovarova, Yulia; Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria); Robinson, Derrick (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)

Trypanosoma brucei belongs to a group of unicellular, flagellated parasites that are responsible for human African trypanosomiasis. Its flagellum originates from the mature basal body, exits the cell body through the flagellar pocket (FP) and remains attached to the cell body via the flagellum attachment zone. At the distal end of the FP, an essential intracellular cytoskeletal structure called the flagellar pocket collar (FPC) circumvents the flagellum.We have previously characterised the structure and assembly of a multidomain FPC protein named BILBO1, which serves as an FPC skeleton and for the docking of other FPC components, such as FPC4, to maintain the structure and function of the FPC. We have identified several BILBO1 partners including FPC4 and FPC3. FPC3 shares ~30% sequence identities with BILBO1 within their N-terminal domains (NTD) and colocalises with BILBO1 in vivo. Using complementary approaches including crystallisation, yeast two-hybrid interaction assays and biochemical studies, we have demonstrated that the C-terminal domain of FPC4 interacts with both the BILBO1-NTD and the FPC3-NTD suggesting a common functional role of these NTD domains. We further identify several other BILBO1-NTD like proteins, all of which localise to the basal body-FPC area and interact with FPC4 with similar binding affinities, suggesting a common functional domain present along the path to the FPC or required for FPC targeting.

Author Index

A

Acharyya, Nirmallya 45, 242

Acosta, David (CBER, Food and Drug Administration) 39, 45, 203, 242

Acosta-Serrano, Alvaro (School of Tropical Medicine) 25, 114

Afasizhev, Ruslan (Boston University) 8, 37, 182,

Afasizheva, Inna (Boston University) 35, 164

Afrin, Marjia 10, 35, 52, 172,

Almeida da Silva, Gabriel Lamak 39, 198

Alvarez, Vanina Eder (Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina) 43, 222

Amodeo, Simona (University Bern) 14, 43, 71, 224

AN, TAI (Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston) 14, 27, 74, 118

Aroko, Erick (Würzburg University) 37, 73

Awuah-Mensah, Georgina (University of Nottingham) 40, 221

B

Baker, Nicola (University of York) 15, 27, 77, 118

Bangs, Jay (University of Buffalo) 10, 14, 22, 27, 40, 55, 72, 102, 118, 212,

Beaver, Alexander (Johns Hopkins School of Medicine) 44, 235

Bellofatto, Vivian (Rutgers- New Jersey Medical School)

Beneke, Tom (University of Oxford, Sir William Dunn School of Pathology) 17, 81

Beverley, Stephen (Washington University School of Medicine) 25, 46, 110, 252

Bevkal Subramanyaswamy, Shubha (Institute of Cell Biology, University of Bern, Switzerland) 35, 168

Bishola, Tania (ZMBH, UNIVERSITY OF HEIDELBERG) 43, 219

Bonhivers, Melanie (CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France Pathogénicité, UMR 5234, Bordeaux, France) 14, 28, 47, 70, 122, 256

Bower-Lepts, Christopher 46, 247

Brambilla Trindade Carnielli, Juliana (York Biomedical Research Institute, Department of Biology, University of York, UK) 36, 177

Briggs, Emma (WCIP, University of Glasgow) 22, 38, 99, 103, 189

Bringaud, Frederic (Universite Bordeaux Segalen) 12, 22, 25, 33, 36, 58, 59, 101, 112, 155, 179

Brink, Benedikt (LMU Munich) 22, 33, 38, 44, 104, 158, 193, 229

Brusini, Lorenzo (University of Nottingham) 19, 35, 93, 166

Budzak, James (Imperial College) 10, 49

Burge, Rebecca (The University of York) 12, 27, 65, 118

Burleigh, Barbara (Harvard School of Public Health) 12, 61

Butenko, Anzhelika (Institute of Parasitology) 31, 144

Butter, Falk 12, 30, 33, 35, 36, 63, 135, 155, 163, 175

C

Call, Daniel (Brigham Young University) 45, 243

CALVO ALVAREZ, ESTEFANIA (Institut Pasteur) 25, 112, 113

Canavate del Pino, Ricardo 77, 187

Carloni, Roberta (University of Edinburgh) 23, 35, 42, 107, 171, 215

Carnes, Jason (Seattle Children's Research Institute) 30, 139

Carrington, Mark (University of Cambridge, Department of Biochemistry) 15, 17, 27, 28, 40, 75, 84, 86, 118, 123, 124, 209

Carter, Nicola (Pacific University School of Pharmacy, 222 SE 8th Ave, Suit 451, Hillsboro, OR 97123) 43, 223

Carvalho, Sandra

Casas-Sanchez, Aitor (Liverpool School of Tropical Medicine) 25, 114

Castillo, Julio

Cayla, Mathieu (University of Edinburgh) 25, 111

Chaudhuri, Minu (MEHARRY MEDICAL COLLEGE) 32, 154

Christensen, Kenneth (Brigham Young University) 31, 45, 145, 243

Ciganda, Martin (University at Buffalo) 32, 44, 150, 227

Cosentino, Raul (Department of Veterinary Sciences, Experimental Parasitology. Ludwig-Maximilians-Universität München, Germany) 22, 33, 104, 158

Cross, George (The Rockefeller University)

Crouch, Kathryn (University of Glasgow) 22, 29, 37, 38, 43, 99, 103, 130, 184, 189, 221

Crowe, Logan (Clemson University) 31, 148

Cruz-Reyes, Jorge (Texas A&M University) 29, 131

Cunha, Julia (Butantan Institute) 25, 31, 116, 143

D

da Silva, Marcelo 37, 181

Damasceno, Jeziel Dener (University of Glasgow) 19, 22, 29, 40, 92, 99, 130, 208

D'Archivio, Simon (University of Nottingham) 19, 36, 93, 173

Davies, Carys (Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, London) 45, 239

de Freitas Nascimento, Janaína (Universidade de Sao Paulo) 25, 116

de Graffenried, Christopher (Brown University) 19, 36, 94, 178

Dean, Samuel (University of Oxford) 15, 27, 37, 75, 118, 186

Debler, Erik (Rockefeller University)

Delzell, Stephanie (University of Massachusetts, Amherst) 39, 206

Dewar, Caroline 12, 64

Dey, Abhishek (University of North Carolina at Charlotte) 35, 169

DiMaio, John (Penn State Brandywine) 38, 46, 196, 250

Dolezelova, Eva (Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic) 12, 36, 63, 179

Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria) 14, 28, 35, 47, 70, 122, 170, 256

Dumoulin, Peter (Harvard T.H. Chan School of Public Health) 12, 61

Duncan, Samuel (The University of Dundee) 12, 27, 33, 66, 118, 157

E

Ebenezer, Karen (Hunter College CUNY) 28, 125

Echeverry, Maria (Universidad Nacional de Colombia) 46, 248

Eisenhuth, Nicole (Department of Cell and Developmental Biology, Würzburg, Germany) 30, 135

ERBEN, Esteban (DKFZ) 45, 241

F

Faria, Joana (School of Life Sciences, University of Dundee) 10, 50

Fathallah, Nadin (Lancaster University) 29, 133

Fesser, Anna 33, 159

Figueiredo, Luisa (Instituto de Medicina Molecular) 10, 25, 32, 33, 36, 44, 51, 113, 152, 155, 163, 173, 235

Florini, Francesca (IZB, University of Bern, Switzerland) 22, 101

Forrester, Sarah (York Biomedical Research institute, Department of Biology, University of York, United Kingdom) 33, 36, 161, 177

Franco, Carlos 46, 248

G

Gadelha, Catarina (University of Nottingham) 10, 19, 36, 40, 53, 93, 173, 211, 213,

Garrison, Paige (Univeristy at Buffalo) 10, 27, 55, 118

Gaurav, Amit (Cleveland State University) 10, 35, 52, 172

Geoghegan, Vincent (University of York) 15, 19, 27, 42, 77, 95, 118, 215

Glover, Lucy 10, 39, 40, 45, 50, 201, 207, 238

Gosavi, Ujwala (UConn Health) 42, 218

Gritz, Sam

Guegan, Fabien (Instituto de Medicina Molecular) 25, 113

Gull, Keith (University of Oxford) 14, 15, 27, 37, 43, 69, 75, 118, 186, 224

H

Haindrich, Alexander C. (IPS, Univeristy of Bern, Bern, CHE) 29, 128

Hajduk, Stephen (University of Georgia)

Halliday, Clare 15, 27, 75, 118

Hambleton, Isobel (University of Cambridge) 28, 124

Harb, Omar 37, 184

Hashimi, Hassan (Institute of Parasitology, Biology Center AS

CR and University of South Bohemia, Faculty of Science) 21, 121

Hayashi, Hanako (University of Oxford) 19, 89

Heise, Norton (Universidade Federal do Rio de Janeiro) 38, 192

Horakova, Eva (Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceské Budejovice, Czech Republic) 20, 32, 126, 153

Horn, David 10, 12, 23, 29, 42, 45, 50, 60, 106, 127, 215, 237, 249

Hovel-Miner, Galadriel (The George Washington University) 23, 31, 42, 105, 141, 142, 215

Hutchinson, Sebastian (Institut Pasteur) 10, 12, 46, 50, 60, 249

I

Imhof, Simon (UCLA) 14, 68

Iribarren, Paula 43, 222

J

Janzen, Christian (University Wuerzburg) 12, 30, 36, 63, 135, 163, 175

Jaremko, Daniel (University at Buffalo, Jacobs School of Medicine and Biomedical Sciences) 32, 150

Jeffares, Daniel 33, 36, 161, 177

Jensen, Bryan (Seattle Childrens Research Institute) 39, 204

Ji, Zhe 33, 157

Jones, Jessica (Clemson University) 29, 129

K

Kelly, Felice (Oregon Health Sciences University) 46, 253

Khalizova, Nailya 39, 202

Kieft, Rudo (Department of Biochemistry and Molecular Biology, University of Georgia) 22, 98

Kim, Hee-Sook (The Rockefeller University) 23, 42, 105, 215

Klingbeil, Michele (University of Massachusetts, Amherst) 20, 39, 42, 43, 96, 206, 215, 220

Koeller, Carolina (University at Buffalo) 14, 38, 40, 72, 192, 212

Kolev, Nikolay (Yale University) 10, 25, 38, 49, 115, 191

Kovarova, Julie (University of Dundee) 12, 60

Krasilnikova, Marija 43, 221

KRISHNAN, ARUNKUMAR (NIH/NLM/NCBI) 36, 176

L

Lander, Noelia (University of Georgia) 38, 190

Landfear, Scott (Oregon Health & Science University) 12, 46, 62, 253

Lee, Kyu (University of Texas) 31, 148

Li, Bibo (Cleveland State University) 10, 35, 44, 52, 169, 172, 231

Li, Ziyin (University of Texas Medical School at Houston) 14, 27, 30, 31, 36, 74, 118, 138, 146, 174

Licon, Haley (Oregon Health and Science University) 16, 62

Liu, Bin 43, 45, 219, 240

Lukes, Julius (Biology Center) 28, 126

Luzak, Vanessa (LMU Munich) 38, 44, 193, 229

Lyda, Todd (Para Tryp Research Organization)

M

MacGregor, Paula (University of Cambridge) 17, 25, 28, 31, 84, 111, 123, 147

Macleod, Olivia (University of Cambridge) 17, 28, 84, 86, 123

Malfara, Madeline 38, 46, 196, 250

Marques, Catarina (Wellcome Centre for Integrative Parasitology, University of Glasgow) 40, 208

Martel, Daniel 33, 156

Matthews, Keith (University of Edinburgh) 10, 23, 25, 35, 38, 42, 44, 54, 107, 111, 171, 194, 215, 226, 232

McCulloch, Richard (University of Glasgow) 19, 22, 29, 37, 38, 39, 40, 43, 92, 99, 103, 130, 181, 189, 198, 208, 213, 221

McDermott, Suzanne (Seattle Children's Research Institute) 29, 30, 131, 136, 139

McFarland, Madalyn

McLaughlin, Emilia (Institut Pasteur Paris) 39, 201

McWilliam, Kirsty (Ludwig-Maximilians-Universität München) 10, 54

Meehan, Joshua 29, 131

Michaeli, Shulamit (Bar-Ilan University) 22, 30, 100, 137

Moloney, Nicola (University of Cambridge) 31, 147

Monte-Neto, Rubens 33, 160

Moretti, Nilmar (Federal University of Sao Paulo) 30, 46, 134, 254

Morris, James (Clemson University) 29, 45, 129, 243

Mosquillo, Florencia (Facultad de Ciencias, Universidad de la República) 37, 188

Mottram, Jeremy (University of York) 12, 14, 15, 19, 27, 33, 36, 38, 42, 45, 46, 65, 69, 77, 88, 95, 118, 161, 177, 189, 215, 244, 247

Mugnier, Monica (Johns Hopkins Bloomberg School of Public Health) 10, 27, 39, 44, 54, 56, 118, 199, 235

Myler, Peter (Seattle Biomedical Research Institute)

N

Neish, Rachel 15, 27, 45, 77, 118, 244

Nievas, Romina

Nkouawa, Agathe (Yale School of Public Health) 25, 38, 115, 191

O

Ochsenreiter, Torsten (University of Bern) 14, 43, 71, 224

Ooi, Cher-Pheng (Imperial College) 40, 209

Oprea, Yasmine (CUNY Hunter College) 46, 251

P

Padilla-Mejia, Norma E (University of Dundee) 27, 119

Pant, Jyoti (The Graduate Center, City University of New York) 37, 185

Paul, Kimberly (Clemson University) 40, 43, 210, 225

Pedroso dos Santos, Gregory 30, 134

Pivovarova, Yulia (Medical University of Vienna) 14, 47, 70, 256

Podesvova, Lucie (University of Ostrava, Faculty of Science, Life Science Research Centre, Czech Republic) 46, 246

Poudyal, Nava (Clemson University) 43, 225

Povelones, Megan (Penn State Brandywine) 17, 38, 46, 82, 196, 250

Q

Quinn, McKenzie (The George Washington University) 23 ,31, 42, 105, 142, 215

Quintana, Juan (University of Dundee) 37, 180

R

Rabuffo, Claudia (LMU Munich) 45, 238

Rachidi, Najma (Institut Pasteur) 32, 33, 151, 156

Raja, Sripriya 44, 233

Ramirez-Barrios, Roger (Department of Biomedical Sciences, University of Minnesota Medical School, Duluth campus, Duluth, Minnesota, USA.) 42, 216

Ramsdell, Trisha

Read, Laurie (Wade J. Sigurdson) 30, 39 ,140 205

Reuter, Christian 17, 83

Rico, Eva (Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK) 29, 127

Roditi, Isabel (Institute of Cell Biology, University of Bern) 17, 22, 35, 79, 80, 101, 168

Rojas Sanchez, Saul (Yale School of Public Health) 44, 228

Romero-Meza, Gabriela (Johns Hopkins Bloomberg School of Public Health) 10, 27, 56, 118

Roos, David (Univ Pennsylvania) 37, 184

Rudenko, Gloria (Imperial College London) 10, 40, 45, 49, 209, 239

S

Sabatini, Robert (University of Georgia) 22, 98

Saliutama, Joshua (Eukaryotic Pathogens Innovation Center, Clemson University) 40, 210

Sanches-Vaz, Margarida (Instituto de Medicina Molecular) 32, 152

Santi, Ana Maria (Washington University School of Medicine in St. Louis and René Rachou Institute, FIOCRUZ MINAS) 46, 252

Savran, Michelle (Hunter College CUNY) 46, 255

Scheidt, Viktor 45, 237

Schenkman, Sergio (Universidade Federal de Sao Paulo) 30, 38, 46, 134, 197, 254

Schulz, Danae (Harvey Mudd College) 28, 120

Shapira, Michal (Ben-Gurion University of the Negev) 20, 42, 108, 215

Sharif, Mohamed

Shaw, Sebastian (Institute of Cell Biology) 17, 80

Shi, Huafang (Yale school of medicine) 25, 38, 115, 191

Shirinyan-Tuka, Lilit 39, 202

Shlomai, Joseph (Department of Microbiology and Molecular Genetics The Hebrew University of Jerusalem) 35, 167

Siegel, Nicolai (LMU Munich) 22, 33, 38, 44, 104, 158, 193, 229

SIMA TERUEL, NURIA 40, 207

Sinclair, Amy (Brown University) 36, 178

SINGHA, UJJAL (MEHARRY MEDICAL COLLEGE) 32, 154

Smircich, Pablo (Instituto de Investigaciones Biológicas Clemente Estable / Facultad de Ciencias) 37, 44, 188, 230

Smith, Jaclyn

Smith Jr., Joseph 32, 39, 154, 205

So, Jaime (Johns Hopkins Bloomberg School of Public Health) 39, 199

Soni, Awakash (Hebrew University, Jerusalem, Israel) 35, 167

SOUZA-MELO, NORMANDA (FEDERAL UNIVERSITY OF SAO PAULO) 38, 197

Springer, Amy (UMass Amherst)

Srivastava, Ankita (Department of Genetics and Genome Sciences, UConn Health, Farmington, CT) 38, 195

Staneva, Desislava (The University of Edinburgh) 23, 35, 42, 107, 171, 215

Stepinac, Emma (Max F. Perutz Laboratories, Medical University of Vienna) 28, 122

Sterkers, Yvon (University Montpellier) 19, 91

Stuart, Ken 29, 30, 131, 138, 139

Subota, Ines (LMU Munich) 38, 193

Sunter, Jack (Oxford Brookes University) 14, 15, 27, 69, 75, 118

T

Takagi, Yuko (National Institute of Advanced Industrial Science and Technology (AIST), Japan) 42, 217

Tettey, Mabel Deladem (School of Biological Sciences, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland) 38, 194

Tiengwe, Calvin (Imperial College London) 22, 40, 102, 212

Tihon, Eliane 45, 236

Toh, Justin (Yale School of Public Health) 25, 38, 115, 191

Tosi, Luiz (Universidade de Sao Paulo) 19, 38, 39, 92, 189, 198

Trenaman, Anna (University of Dundee) 23, 42, 108, 215

Trevor, Camilla (University of Cambridge) 17, 84, 86

Tschudi, Christian (Yale School of Public Health) 22, 29, 38, 44, 49, 100, 115, 191, 228

Tylec, Brianna (University at Buffalo) 30, 39, 140, 205

U

Urbaniak, Michael (Lancaster University) 19, 29, 39, 90, 113, 201

V

van Luenen, Henri (The Netherlands Cancer Institute)

Vance, Jacob (Brigham Young University) 31, 45, 145, 243

Venter, Frank 44, 226

Verdi, Joey (Hunter College, City University of New York) 17, 28, 37, 46, 85, 125, 185, 251, 255

Viegas, Idalio (Instituto de Medicina Molecular) 10, 51

Villafraz, Oriana (MFP (CNRS UMR-5234), University of Bordeaux, Bordeaux, France) 12, 59

W

Wallin, Stephen 31, 141

Warren, Felix 40, 213

Weisert, Nadine (Department of Cell and Developmental Biology, Würzburg, Germany) 36, 175

Wheeler, Richard (University of Oxford) 15, 17, 27, 75, 76, 81, 118

Wickstead, Bill (University of Nottingham) 19,, 35, 36, 40, 45, 93, 166, 173, 211, 239

Wilkinson, Christina 31, 45, 148, 245

Williams, Noreen (University of Buffalo) 32, 44, 150, 227

Y

Yamada, Kayo (University of Dundee) 29, 37, 132, 200

Yuan, Xiaojing

Yurchenko, Vyacheslav (Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic) 28, 35, 46, 126, 165, 246

Z

Zhang, Kai (Texas Tech University)

Zhou, Qing (University of Texas Medical School at Houston) 14, 27, 30, 36, 74, 118, 138, 174

Zikova, Alena (Biology Centre) 12, 63

Zimmer, Sara (University of Minnesota Medical School) 42, 216

Nirmallya Acharyya
nirmallya.acharyya@fda.hhs.gov

David Acosta
CBER, Food and Drug Administration
david.acosta@fda.hhs.gov

Alvaro Acosta-Serrano
School of Tropical Medicine
alvaro.acosta-serrano@lstmed.ac.uk

Ruslan Afasizhev
Boston University
ruslana@bu.edu

Inna Afasizheva
Boston University
innaaf@bu.edu

Marjia Afrin
m.afrin@vikes.csuohio.edu

Gabriel Lamak Almeida da Silva
gabriellamak93@usp.br

Vanina Eder Alvarez
Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, (1650) Buenos Aires, Argentina
valvarez@iib.unsam.edu.ar

Simona Amodeo
University Bern
simona.amodeo@izb.unibe.ch

TAI AN
Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston tai.an@uth.tmc.edu

Erick Aroko
Würzburg University
eric.aroko@gmail.com

Georgina Awuah-Mensah
University of Nottingham
georgina.awuah-mensah@nottingham.ac.uk

Nicola Baker
University of York
nicola.baker@york.ac.uk

Jay Bangs
University of Buffalo
jdbangs@buffalo.edu

Alexander Beaver
Johns Hopkins School of Medicine
abeaver3@jhmi.edu

Vivian Bellofatto
Rutgers- New Jersey Medical School bellofat@njms.rutgers.edu

Tom Beneke
University of Oxford, Sir William Dunn School of Pathology
tom.beneke@path.ox.ac.uk

Stephen Beverley
Washington University School of Medicine
beverley@wusm.wustl.edu

Shubha Bevkal Subramanyaswamy
Institute of Cell Biology, University of Bern, Switzerland
shubha.bevkal@izb.unibe.ch

Tania Bishola
ZMBH, UNIVERSITY OF HEIDELBERG
taniabishola@gmail.com

Melanie Bonhivers
CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France
Pathogénicité, UMR 5234, Bordeaux, France
melanie.bonhivers@u-bordeaux.fr

Christopher Bower-Lepts
chris.bower-lepts@york.ac.uk

Juliana Brambilla Trindade Carnielli
York Biomedical Research Institute, Department of Biology, University of York, UK
julianacarnielli@gmail.com

Emma Briggs
WCIP, University of Glasgow
emma.briggs.2@glasgow.ac.uk

Frederic Bringaud
Universite Bordeaux Segalen

Benedikt Brink
LMU Munich
b.brink@lmu.de

Lorenzo Brusini
University of Nottingham
mbxlb1@nottingham.ac.uk

James Budzak
Imperial College
j.budzak14@imperial.ac.uk

Rebecca Burge
The University of York
rjb595@york.ac.uk

Barbara Burleigh
Harvard School of Public Health
bburleig@hsph.harvard.edu

Anzhelika Butenko
Institute of Parasitology
anzhelika.i.butenko@gmail.com

Falk Butter
f.butter@imb.de

Daniel Call
Brigham Young University
kf7jxb@gmail.com

ESTEFANIA CALVO ALVAREZ
Institut Pasteur estefania.calvo-alvarez@pasteur.fr

Ricardo Canavate del Pino
r.canavatedelpino@dundee.ac.uk

Roberta Carloni
University of Edinburgh
roberta.carloni@ed.ac.uk

Jason Carnes
Seattle Children's Research Institute
jason.carnes@seattlechildrens.org

Mark Carrington
University of Cambridge, Department of Biochemistry
mc115@cam.ac.uk

Nicola Carter
Pacific University School of Pharmacy, 222 SE 8th Ave, Suit 451, Hillsboro, OR 97123
cartern@pacificu.edu

Sandra Carvalho
szcarvalho@dundee.ac.uk

Aitor Casas-Sanchez
Liverpool School of Tropical Medicine aitor.casas-sanchez@lstmed.ac.uk

Julio Castillo
juliocastillo25@gmail.com

Mathieu Cayla
University of Edinburgh
mathieu.cayla@ed.ac.uk

Minu Chaudhuri
MEHARRY MEDICAL COLLEGE
mchaudhuri@mmc.edu

Kenneth Christensen
Brigham Young University
kenc@chem.byu.edu

Martin Ciganda
University at Buffalo
mciganda@buffalo.edu

Raul Cosentino
Department of Veterinary Sciences, Experimental Parasitology. Ludwig-Maximilians-Universität München, Germany
rulicosentino@gmail.com

George Cross
The Rockefeller University
george.cross@rockefeller.edu

Kathryn Crouch
University of Glasgow
kathryn.crouch@glasgow.ac.uk

Logan Crowe
Clemson University
lpcrowe@clemson.edu

Jorge Cruz-Reyes
Texas A&M University
cruzrey@tamu.edu

Julia Cunha
Butantan Institute
julia.cunha@butantan.gov.br

Simon D Archivio
University of Nottingham
plzsad@nottingham.ac.uk

Marcelo da Silva
mamasantos2003@yahoo.com.br

Jeziel Dener Damasceno
University of Glasgow
jezielbqi@gmail.com

Carys Davies
Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, London
cd1715@ic.ac.uk

Janaína de Freitas Nascimento
Universidade de Sao Paulo
janainafn@usp.br

Christopher de Graffenried
Brown University
christopher_degraffenried@brown.edu

Samuel Dean
University of Oxford
samuel.dean@path.ox.ac.uk

Erik Debler
Rockefeller University
erik.debler@jefferson.edu

Stephanie Delzell
University of Massachusetts, Amherst sdelzell@umass.edu

Caroline Dewar
caroline.dewar@dcb.unibe.ch

Abhishek Dey
University of North Carolina at Charlotte adey2@uncc.edu

John DiMaio
Penn State Brandywine
jcd5275@psu.edu

Eva Dolezelova
Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic
dolezelova@paru.cas.cz

Gang Dong
Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria
gang.dong@meduniwien.ac.at

Peter Dumoulin
Harvard T.H. Chan School of Public Health
peter.dumoulin@gmail.com

Samuel Duncan
The University of Dundee
svduncan@dundee.ac.uk

Karen Ebenezer
Hunter College CUNY
karen5ebenezer@gmail.com

Maria Echeverry
Universidad Nacional de Colombia
mcecheverryg@unal.edu.co

Nicole Eisenhuth
Department of Cell and Developmental Biology, Würzburg, Germany
nicole.eisenhuth@uni-wuerzburg.de

Esteban ERBEN
DKFZ
e.erben@dkfz-heidelberg.de

Joana Faria
School of Life Sciences, University of Dundee
j.r.correiafaria@dundee.ac.uk

Nadin Fathallah
Lancaster University
nadinfathallah@gmail.com

Anna Fesser
anna.fesser@swisstph.ch

Luisa Figueiredo
Instituto de Medicina Molecular
lmf@medicina.ulisboa.pt

Francesca Florini
IZB, University of Bern, Switzerland
francesca.florini@izb.unibe.ch

Sarah Forrester
York Biomedical Research institute, Department of Biology, University of York, United Kingdom
sarah.forrester@york.ac.uk

Carlos Franco
cefrancom@unal.edu.co

Catarina Gadelha
University of Nottingham
catarina.gadelha@nottingham.ac.uk

Paige Garrison
Univeristy at Buffalo
pd32@buffalo.edu

Amit Gaurav
Cleveland State University
amitgaurav23@gmail.com

Vincent Geoghegan
University of York
vincent.geoghegan@york.ac.uk

Lucy Glover
lucy.glover@pasteur.fr

Ujwala Gosavi
UConn Health
ujugosavi@gmail.com

Sam Gritz
gritz122@gmail.com

Fabien Guegan
Instituto de Medicina Molecular
fguegan@fm.ul.pt

Keith Gull
University of Oxford
keith.gull@path.ox.ac.uk

Alexander C. Haindrich
IPS, Univeristy of Bern,
Bern, CHE
alexander.haindrich@ips.unibe.ch

Stephen Hajduk
University of Georgia
shajduk@bmb.uga.edu

Clare Halliday
18026742@brookes.ac.uk

Isobel Hambleton
University of Cambridge
ih291@cam.ac.uk

Omar Harb
oharb@upenn.edu

Hassan Hashimi
Institute of Parasitology, Biology Center AS CR and University of South Bohemia, Faculty of Science hassan@paru.cas.cz

Hanako Hayashi
University of Oxford
hanako.hayashi@bioch.ox.ac.uk

Norton Heise
Universidade Federal do Rio de Janeiro nheise@biof.ufrj.br

Eva Horakova
Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceské Budejovice, Czech Republic
horakova@paru.cas.cz

David Horn
d.horn@dundee.ac.uk

Galadriel Hovel-Miner
The George Washington University
ghovel_miner@gwu.edu

Sebastian Hutchinson
Institut Pasteur
sebastian.hutchinson@pasteur.fr

Simon Imhof
UCLA
simon.imhof1981@gmail.com

Paula Iribarren
piribarren@iibintech.com.ar

Peter J Myler
Seattle Biomedical Research Institute

Christian Janzen
University Wuerzburg
christian.janzen@uni-wuerzburg.de

Daniel Jaremko
University at Buffalo, Jacobs School of Medicine and Biomedical Sciences
danielja@buffalo.edu

Daniel Jeffares
daniel.jeffares@york.ac.uk

Bryan Jensen
Seattle Childrens Research Institute
bryan.jensen@seattlechildrens.org

Zhe Ji
z.ji@dundee.ac.uk

Jessica Jones
Clemson University
jjone29@g.clemson.edu

Felice Kelly
Oregon Health Sciences University
felice.kelly@gmail.com

Nailya Khalizova
nailya368@gmail.com

Rudo Kieft
Department of Biochemistry and Molecular Biology, University of Georgia doffo001@uga.edu

Hee-Sook Kim
The Rockefeller University
kheesook10@gmail.com

Michele Klingbeil
University of Massachusetts, Amherst
klingbeil@microbio.umass.edu

Carolina Koeller
University at Buffalo
ckoeller@buffalo.edu

Nikolay Kolev
Yale University
nikolay.kolev@yale.edu

Julie Kovarova
University of Dundee
j.kovarova@dundee.ac.uk

Marija Krasilnikova
m.krasilnikova.1@research.gla.ac.uk

ARUNKUMAR KRISHNAN
NIH/NLM/NCBI
arunkumar.krishnan@nih.gov

Noelia Lander
University of Georgia
noelia@uga.edu

Scott Landfear
Oregon Health & Science University
landfear@ohsu.edu

Kyu Lee
University of Texas
kyu.j.lee@uth.tmc.edu

Bibo Li
Cleveland State University
b.li37@csuohio.edu

Ziyin Li
University of Texas Medical School at Houston
ziyin.li@uth.tmc.edu

Haley Licon
Oregon Health and Science University licon@ohsu.edu

Bin Liu
233345754@qq.com

Julius Lukes
Biology Center
jula@paru.cas.cz

Vanessa Luzak
LMU Munich
vanessa.luzak@gmail.com

Todd Lyda
Para Tryp Research Organization
todd.a.lyda@paratryp.org

Paula MacGregor
University of Cambridge
pm560@cam.ac.uk

Olivia Macleod
University of Cambridge
ojsm2@cam.ac.uk

Madeline Malfara
mfm5553@psu.edu

Catarina Marques
Wellcome Centre for Integrative Parasitology, University of Glasgow
catarina.dealmeidamarques@glasgow.ac.uk

Daniel Martel
daniel.martel@pasteur.fr

Keith Matthews
University of Edinburgh
keith.matthews@ed.ac.uk

Richard McCulloch
University of Glasgow
richard.mcculloch@glasgow.ac.uk

Suzanne McDermott
Seattle Children's Research Institute
suzanne.mcdermott@seattlechildrens.org

Madalyn McFarland
mcfarland@g.harvard.edu

Emilia McLaughlin
Institut Pasteur Paris
ejmclaughlin@live.co.uk

Kirsty McWilliam
Ludwig-Maximilians-Universität München
k.r.mcwilliam@sms.ed.ac.uk

Joshua Meehan
joshua.meehan1@tamu.edu

Shulamit Michaeli
Bar-Ilan University
shulamit.michaeli@biu.ac.il

Nicola Moloney
University of Cambridge
nm657@cam.ac.uk

Rubens Monte-Neto
rubensdomonte@gmail.com

Nilmar Moretti
Federal University of Sao Paulo
nilmarsmoretti@gmail.com

James Morris
Clemson University
jmorri2@clemson.edu

Florencia Mosquillo
Facultad de Ciencias, Universidad de la República
mmosquillo@fcien.edu.uy

Jeremy Mottram
University of York
jeremy.mottram@york.ac.uk

Monica Mugnier
Johns Hopkins Bloomberg School of Public Health
mmugnie1@jhu.edu

Rachel Neish
rn745@york.ac.uk

Romina Nievas
romina.nievas@york.ac.uk

Agathe Nkouawa
Yale School of Public Health
agathe.nkouawa@yale.edu

Torsten Ochsenreiter
University of Bern
torsten.ochsenreiter@izb.unibe.ch

Cher-Pheng Ooi
Imperial College
cher.ooi@imperial.ac.uk

Yasmine Oprea
CUNY Hunter College
yasmine.oprea00@myhunter.cuny.edu

Norma E Padilla-Mejia
University of Dundee
nepadillamejia@dundee.ac.uk

Jyoti Pant
The Graduate Center, City University of New York
jpanta@genectr.hunter.cuny.edu

Kimberly Paul
Clemson University
kpaul@clemson.edu

Gregory Pedroso dos Santos
gregpesan@gmail.com

Yulia Pivovarova
Medical University of Vienna
yulia.pivovarova@univie.ac.at

Lucie Podesvova
University of Ostrava, Faculty of Science, Life Science Research Centre, Czech Republic
podesvoval@email.cz

Nava Poudyal
Clemson University
npoudya@g.clemson.edu

Megan Povelones
Penn State Brandywine
mlp29@psu.edu

McKenzie Quinn
The George Washington University quinnmr@gwu.edu

Juan Quintana
University of Dundee
jfzquintanaalcala@dundee.ac.uk

Claudia Rabuffo
LMU Munich
c.rabuffo@lmu.de

Najma Rachidi
Institut Pasteur
najma.rachidi@pasteur.fr

Sripriya Raja
sraja@g.clemson.edu

Roger Ramirez-Barrios
Department of Biomedical Sciences, University of Minnesota Medical School, Duluth campus, Duluth, Minnesota, USA.
rramirez@d.umn.edu

Trisha Ramsdell
trisha.ramsdell@yale.edu

Laurie Read
Wade J. Sigurdson
lread@buffalo.edu

Christian Reuter
christian.reuter@uni-wuerzburg.de

Eva Rico
Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK
e.ricovidal@dundee.ac.uk

Isabel Roditi
Institute of Cell Biology, University of Bern isabel.roditi@izb.unibe.ch

Saul Rojas Sanchez
Yale School of Public Health
saul.rojas-sanchez@yale.edu

Gabriela Romero-Meza
Johns Hopkins Bloomberg School of Public Health
gromero5@jhu.edu

David Roos
Univ Pennsylvania
droos@sas.upenn.edu

Gloria Rudenko
Imperial College London
gloria.rudenko@imperial.ac.uk

Robert Sabatini
University of Georgia

Joshua Saliutama
Eukaryotic Pathogens Innovation Center, Clemson University
jsaliut@g.clemson.edu

Margarida Sanches-Vaz
Instituto de Medicina Molecular
amargaridavaz@medicina.ulisboa.pt

Ana Maria Santi
Washington University School of Medicine in St. Louis and René Rachou Institute, FIOCRUZ MINAS
anamms552@gmail.com

Michelle Savran
Hunter College CUNY
mjs654@cornell.edu

Viktor Scheidt
v.scheidt@dundee.ac.uk

Sergio Schenkman
Universidade Federal de Sao Paulo
sschenkman@unifesp.br

Danae Schulz
Harvey Mudd College
dschulz@hmc.edu

Michal Shapira
Ben-Gurion University of the Negev
shapiram@bgu.ac.il

Mohamed Sharif
masharif@buffalo.edu

Sebastian Shaw
Institute of Cell Biology
sebastian.millius@izb.unibe.ch

Huafang Shi
Yale school of medicine
huafang.shi@yale.edu

Lilit Shirinyan-Tuka
lilooshik@gmail.com

Joseph Shlomai
Department of Microbiology and Molecular Genetics The Hebrew University of Jerusalem
josephs@ekmd.huji.ac.il

Nicolai Siegel
LMU Munich
n.siegel@lmu.de

NURIA SIMA TERUEL
nuria.sima-teruel@pasteur.fr

Amy Sinclair
Brown University
amy_sinclair@brown.edu

UJJAL SINGHA MEHARRY MEDICAL COLLEGE
usingha@mmc.edu

Pablo Smircich
Instituto de Investigaciones Biológicas Clemente Estable / Facultad de Ciencias
psmircich@fcien.edu.uy

Jaclyn Smith
jsmit380@jhmi.edu

Joseph Smith Jr.
jsmith85@buffalo.edu

Jaime So
Johns Hopkins Bloomberg School of Public Health
jso4@jhmi.edu

Awakash Soni
Hebrew University, Jerusalem, Israel
soni.awakash@mail.huji.ac.il

NORMANDA SOUZA-MELO
FEDERAL UNIVERSITY OF SAO PAULO
normandasouzamelo@gmail.com

Amy Springer
UMass Amherst
aspringer@biochem.umass.edu

Ankita Srivastava
Department of Genetics and Genome Sciences, UConn Health, Farmington, CT
ankita26@gmail.com

Desislava Staneva
The University of Edinburgh
desislava.staneva@yahoo.com

Emma Stepinac
Max F. Perutz Laboratories,
Medical University of Vienna
emma.stepinac@univie.ac.at

Yvon Sterkers
University Montpellier
yvon.sterkers@umontpellier.fr

Ken Stuart
ken.stuart@seattlechildrens.org

Ines Subota
LMU Munich
ines.subota@lmu.de

Jack Sunter
Oxford Brookes University
jsunter@brookes.ac.uk

Yuko Takagi
National Institute of Advanced
Industrial Science and Technology
(AIST), Japan yuko-
takagi@aist.go.jp

Mabel Deladem Tettey
School of Biological Sciences,
Ashworth Laboratories, University
of Edinburgh, West Mains Road,
Edinburgh EH9 3JT, Scotland
mabel.tettey@ed.ac.uk

Calvin Tiengwe
Imperial College London
c.tiengwe@imperial.ac.uk

Eliane Tihon
eliane.tihon@pasteur.fr

Justin Toh
Yale School of Public Health
justin.toh@yale.edu

Luiz Tosi
Universidade de Sao Paulo
luiztosi@fmrp.usp.br

Anna Trenaman
University of Dundee
a_trenaman@hotmail.com

Camilla Trevor
University of Cambridge
ct504@cam.ac.uk

Christian Tschudi
Yale School of Public Health
christian.tschudi@yale.edu

Brianna Tylec
University at Buffalo
tylecbrianna@gmail.com

Michael Urbaniak
Lancaster University
m.urbaniak@lancaster.ac.uk

Henri van Luenen
The Netherlands Cancer Institute
h.v.luenen@nki.nl

Jacob Vance
Brigham Young University
jacobav96@gmail.com

Frank Venter
f.venter@ed.ac.uk

Joey Verdi
Hunter College,
City University of New York
joseph.verdi05@gmail.com

Idalio Viegas
Instituto de Medicina Molecular
iviegas@fm.ul.pt

Oriana Villafraz
MFP (CNRS UMR-5234),
University of Bordeaux, Bordeaux,
France oriana.villafraz-
marquez@u-bordeaux.fr

Stephen Wallin
stwallin@gwu.edu

Felix Warren
f.warren.1@research.gla.ac.uk

Nadine Weisert
Department of Cell and
Developmental Biology,
Würzburg, Germany
nadine.weisert@uni-wuerzburg.de

Richard Wheeler
University of Oxford
richard.wheeler@path.ox.ac.uk

Bill Wickstead
University of Nottingham
bill.wickstead@nottingham.ac.uk

Christina Wilkinson
cwilki7@g.clemson.edu

Noreen Williams
University of Buffalo
nw1@acsu.buffalo.edu

Kayo Yamada
University of Dundee
k.ono@dundee.ac.uk

Xiaojing Yuan
xyuan12@umd.edu

Vyacheslav Yurchenko
Life Science Research Centre,
University of Ostrava, Ostrava,
Czech Republic
vyacheslav.yurchenko@osu.cz

Kai Zhang
Texas Tech University
kai.zhang@ttu.edu

Qing Zhou
University of Texas Medical
School at Houston
qing.zhou@uth.tmc.edu

Alena Zikova
Biology Centre
azikova@paru.cas.cz

Sara Zimmer
University of Minnesota Medical
School szimmer3@d.umn.edu