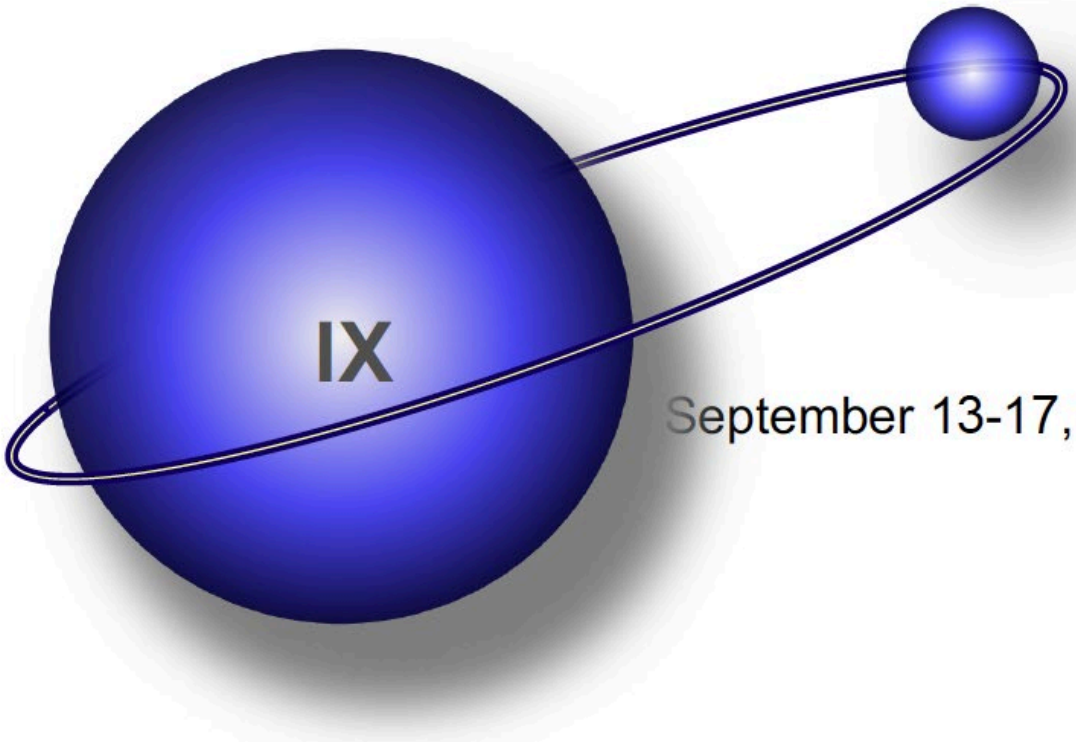
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory . Woods Hole . MA .



September 13-17, 2022

Ninth Kinetoplastid Molecular Cell Biology Meeting

September 13th – 17th, 2022

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

The main motivation for this meeting, independent of the long-running Molecular Parasitology Meeting, is to provide a forum for everyone working on or interested in the Molecular Cell Biology of Trypanosomes and Leishmania and related model organisms.

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

Organizers

2005-2011	**George A.M. Cross**
2013-2019	**Christian Tschudi**
2022	**Christian Tschudi and Luísa M. Figueiredo**





Congratulations to Dyann Wirth and Keith Matthews for receiving the 2023 Alice and C. C. Wang award in Molecular Parasitology

The Alice and C.C. Wang Award recognizes established investigators who are making seminal contributions to the field of molecular parasitology at the time of the award. Novel and significant discoveries on the biology of parasitic organisms are of particular emphasis.

https://www.asbmb.org/career-resources/awards-grants-fellowships/wang

In Memoriam: Dr. Michael Gottlieb, 1944-2021



Michael Gottlieb received his PhD in Biology from the City University of New York and conducted postdoctoral research in the Bacterial Physiology Unit at Harvard Medical School, and the Laboratory of Parasitic Diseases at NIAID/NIH. In 1978, he joined the faculty of the Johns Hopkins University School of Hygiene and Public Health, where his research focused on the biochemistry and cell biology of pathogenic kinetoplastida. Michael was also a member of the NIH Tropical Medicine and Parasitology Study Section from 1988-91. Having risen from Assistant to Associate Professor at Hopkins, he returned to NIAID in 1991 as a program officer in the Parasitology and International Programs Branch, DMID, where he managed the parasite biology grant portfolio, becoming Branch Chief in 2001. Ever mindful of the need for genuine dialog between laboratory-based and field research in global health, he coordinated NIAID's International Centers for Tropical Disease Research network and represented the Institute in its support of the Multilateral Initiative on Malaria. Michael was also responsible for initiating and developing the Institute's pathogen genomics research program, for which he received the Department of Health and Human Service Secretary's Award. Gottlieb played a substantial role in creating an enabling environment and forward-looking translational research program to capitalize on findings in biochemistry, genetics, and genomics, accelerating the development of molecular diagnostics and novel therapeutic interventions for parasitic diseases.

As chief of the Parasitology & International Programs Branch at NIAID, Michael was a strong supporter of the Molecular Parasitology Meetings, and an occasional MPM attendee. He was also instrumental in coordinating multiple funders, institutions, and investigators in support of the *Plasmodium falciparum* genome sequencing project, which was conducted and completed in parallel with the human genome project. Recognizing that genome information and large-scale datasets yet-to-come would need to be archived and made available to the community, Michael chaired or co-chaired the Scientific Advisory Board of the *Plasmodium* Genome Database and its successor projects for 15 years and was instrumental in encouraging the Gates Foundation and Wellcome Trust to support extension of these informatics resources to cover kinetoplastid parasites. By demonstrating cost-effective scalability, this vision pointed the way to further expansion, progressively encompassing other protozoan parasites, pathogenic fungi, arthropod vectors of disease, and the additional taxa now supported by the Eukaryotic Pathogen, Host & Vector Bioinformatics Resource Center (VEuPathDB.org).

Dr. Gottlieb moved to the Foundation for NIH in 2004, as Associate Director for Science, where he was responsible for an array of global health research programs in the areas of vector control, maternal and child health, and drug discovery until his retirement in 2018. Throughout his career, Michael was a model program officer, instrumental in helping many junior scientists navigate the path to independence in the days before formal support and mentorship programs. For more than 30 years, Michael served as a mentor, advisor, and advocate for scientific rigor … but the truly remarkable thing is how universally recognized these attributes are, by the hundreds (maybe thousands) of scientists he counselled. Michael's devotion and exuberance to his work was palpable to all, and it was clear that he gave one hundred percent of himself to his work, his colleagues and to science. He also dedicated one hundred percent of himself to his family. For most people, those percentages don't add up, but for him it did. Michael was a great colleague, mentor, and friend to many of us and will be missed by all.

Also see: https://www.astmh.org/blog/may-2021/in-memoriam-michael-gottlieb,-phd

KMCBM 2022 Acknowledgements

The organizers wish to thank:

James D. Bangs (University at Buffalo, Buffalo, USA) for serving on the program committee.

Omar S. Harb (University of Pennsylvania, Philadelphia, USA) for superb logistical support.

American Society of Tropical Medicine and Hygiene (ASTMH):

ASTMH with support from the Burroughs Wellcome fund for providing travel awards for three KMCBM speakers to attend the ASTMH 2022 Annual Meeting, to be held October 30 – November 3, 2022 in Seattle, Washington.

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 2022 Program

Tuesday September 13th 2022		
2:00PM – 5:00PM	Arrival and registration	Swope
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:00PM	Session I: Cell Biology	Clapp
9:00PM – 11:00PM	Mixer	Swope
Wednesday September 14th 2022		
7:00AM – 8:30AM	Breakfast	Swope
8:45AM – 11:00AM	Session II: Host Pathogen/Drug Discovery	Clapp
12:00PM – 1:30PM	Lunch	Swope
2:00PM – 4:20PM	Session III: Gene Expression I	Clapp
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:00PM	Poster Session A	Swope
9:00PM – 11:00PM	Mixer and posters continued	Swope
Thursday September 15th 2022		
7:00AM – 8:30AM	Breakfast	Swope
8:45AM – 11:00AM	Session IV; Metabolism	Clapp
12:00PM – 1:30PM	Lunch	Swope
12:00PM – 5:00PM	Free time	
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:00PM	Poster Session B	Swope
9:00PM – 11:00PM	Mixer and posters continued	Swope
Friday September 16th 2022		
7:00AM – 8:30AM	Breakfast	Swope
8:45AM – 11:15AM	Session V Gene Expression II	Clapp
12:00PM – 1:30PM	Lunch	Swope
2:00PM – 4:20PM	Session VI: RNA Editing	Clapp
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:00PM	Poster Session C	Swope
9:00PM – 11:00PM	Mixer and posters continued	Swope
Saturday September 17th 2022		
7:00AM – 8:30AM	Breakfast	Swope
9:00AM – 10:30AM	Session VII: Antigenic Variation	Clapp
12:00PM – 1:30PM	Lunch	Swope

******Rooms must be vacated by 10AM on Saturday, unless you have arrangements to stay in the same room for YIP or MPM.***

Abstract book tip: use bookmark navigation in Adobe Acrobat to easily move around.

Session I – Cell Biology

Christopher de Graffenried, Chair
Tuesday 7-9 PM
September 13th 2022

1A The *Trypanosoma cruzi* amastigote flagellum is motile and displays aperiodic beating

Madalyn M Won (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health); Timothy Krüger (Department of Cell- and Developmental Biology, Biozentrum, University of Würzburg); Markus Engstler (Department of Cell- and Developmental Biology, Biozentrum, University of Würzburg); Barbara A Burleigh (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health)

1B cAMP signaling controls adhesion of *Crithidia fasciculata*

Nikki A Holmes (Villanova University); Kelly R Hodges (Villanova University); Madeline F Malfara (Villanova University); Megan L Povelones (Villanova University)

1C Kinetoplastid Attachment Proteins (KAPs): Functional dissection of the *Leishmania* - sand fly attachment interface

Ryuji Yanase (Oxford Brookes University); Flavia Moreira-Leite (Oxford Brookes University); Edward Rea (Oxford Brookes University); Katerina Pruzinova (Charles University); Jovana Sádlová (Charles University); Petr Volf (Charles University); Jack Sunter (Oxford Brookes University)

1D Providing stability without being rigid – the dual nature of the TAC in *Trypanosoma brucei*

Irina Bregy (University of Bern); Julika Radecke (Diamond Light Source); Akira Noga (Paul Scherrer Institute); Mara Kern (University of Bern); Beat Haenni (University of Bern); Takashi Ishikawa (Paul Scherrer Institute); Alistair Siebert (Diamond Light Source); Benoît Zuber (University of Bern); Torsten Ochsenreiter (University of Bern)

1E Structural studies of the ciliary component CFAP410 reveal a bimodular protein and explain how its mutations cause ciliopathies

Alexander Stadler (Medical Univeristy of Vienna); Heloise Gabriel (Oxford Brookes University); Sue Vaughan (Oxford Brookes University); Jack Sunter (Oxford Brookes University); Gang Dong (Medical University of Vienna)

1F A cocktail of VSG-related antigens and digestive enzymes is quantitatively enriched in the African trypanosome secretome

Gustavo Bravo Ruiz (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); Jordan Alexander Forteath (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); Michele Tinti (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); Shravya Murali (The Wellcome Trust

Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); David Horn (University of Dundee)

TT1G The role of ESCRT in endocytosis and secretion of extracellular vesicles trypanosomatids

Nadjania S de L Silva (Unifesp); Suellen R Maran (Unifesp); Nilmar S Moretti (Unifesp); Ana C Torrecilhas (Unifesp); Sergio Schenkman (Unifesp)

TT1H Spatial regulation of the multifunctional dynamin-1-like protein in *Trypanosoma brucei*

Madeline F Malfara (Villanova University); Alexa Pereira (Villanova University); Megan L Povelones (Villanova University)

Session II – Host-Pathogen/Drug Discovery

Keith Gull, Chair
Wednesday 8:45 AM-11:00 AM
September 14th 2022

2A How trypanosomes tell fat to get lost

Henrique Machado (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Peter Hofer (Institute of Molecular Biosciences, University of Graz); Rudolf Zechner (Institute of Molecular Biosciences, University of Graz. BioTechMed-Graz); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

2B Developmental incompetence in selected and naturally occurring trypanosome isolates

Guy Oldrieve (The University of Edinburgh); Mathieu Cayla (The University of Edinburgh); Frederik Van den Broeck (KU Leuven); Keith R Matthews (The University of Edinburgh)

2C Unexpected plasticity in the life cycle of *Trypanosoma brucei*

Jaime N Lisack (Universität Würzburg); Carina Praisler (Universität Würzburg); Sarah Schuster (Universität Würzburg); Markus Engstler (Universität Würzburg)

2D When is a slender not a slender? Assessing terminal commitment to arrest in acute and chronic *Trypanosoma brucei* infections

Stephen D Larcombe (University of Edinburgh); Emma M Briggs (University of Edinburgh); Balazs Szoor (University of Edinburgh); Keith R Matthews (University of Edinburgh)

TT2E A quantitative mass spectrometry approach to identify novel proteins involved in macrophage infections by *Leishmania* parasites

Nicolas Hagedorn (Department of Cell and Developmental Biology, Würzburg, Germany); Albert Fradera Sola (Institue of Molecular Biology, Mainz); Elisa Theres Rauh (Department of Cell and Developmental Biology, Würzburg, Germany); Tobias Gold (Microbiology Institute-Clinical Microbiology, Erlangen); Ulrike Schleicher (Microbiology Institute-Clinical Microbiology, Erlangen); Falk Butter (Institue of Molecular Biology, Mainz); Christian J. Janzen (Department of Cell and Developmental Biology, Würzburg, Germany)

TT2F Trypanosome sequestration and immune response drive acute cerebral disease

Sara Silva Pereira (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Mariana De Niz (Institut Pasteur); Karine Serre (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Joana Coelho (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Marie Ouarné (Instituto de

Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa "); Cláudio A Franco (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

TT2G Development of a molecular diagnostic toolbox for the detection of trypanosomatid parasites by SHERLOCK

Elena Pérez-Antón (Institut Pasteur, Université Paris Cité); Roger Junior Eloiflin (Institut de Recherche pour le Développement (IRD)-CIRAD); Annick Dujeancourt-Henry (Institut Pasteur, Université Paris Cité); Aïssata Camara (Institut Pasteur of Guinea); Jean-Mathieu Bart (Institut de Recherche pour le Développement (IRD)-CIRAD); Brice Rotureau (Institut Pasteur, Université Paris Sorbonne; and Institut Pasteur of Guinea); Lucy Glover (Institut Pasteur, Université Paris Cité)

TT2H Fexinidazole survival genes implicate novel redox pathway in drug resistance

Arthur Drouaud (George Washington University); Jenna Murrow (George Washington University); Galadriel Hovel-Miner (George Washington University)

TT2I A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease

Tamara Sternlieb (Institute of Parasitology, McGill University); Rhiannon Heslop (Institute of Parasitology, McGill University); Mira Loock (Institute of Parasitology, McGill University); Luisa Berenguer Antunes (Institute of Parasitology, McGill University); Igor Cestari (Institute of Parasitology, McGill University;Division of Experimental Medicine, McGill University)

TT2J Structure-guided discovery of a small molecule inhibitor targeting *Trypanosoma brucei* Replication Protein A1

Aditi Mukherjee (Rutgers University, PHRI); Zakir Hossain (Queens College); Junyong Choi (CUNY Graduate Center, Queens College); Hee-Sook Kim (Rutgers University, PHRI)

Session III – Gene Expression I

Monica Mugnier, Chair
Wednesday 2 PM-4:20 PM
September 14th 2022

3A Modified length of the tRNA Trp anticodon stem enabled the UGA stop codon reassignment across species.

Ambar Kachale (Institute of Parasitology, Ceske Budejovice); Zuzana Pavlíková (Czech Academy of Sciences, Prague); Anna Nenarokova (Institute of Parasitology, Ceske Budejovice); Ignacio M. Durante (Institute of Parasitology, Ceske Budejovice); Adriana Roithová (Czech Academy of Sciences, Prague); Petra Miletínová (Czech Academy of Sciences, Prague); Kristína Záhonová (Institute of Parasitology, Ceske Budejovice; Charles University, BIOCEV, Prague); Serafim Nenarokov (Institute of Parasitology, Ceske Budejovice; , Charles University, BIOCEV, Prague); Jan Votýpka, (Institute of Parasitology, Ceske Budejovice; Charles University, BIOCEV, Prague); Eva Horáková (Institute of Parasitology, Ceske Budejovice); Vyacheslav Yurchenko (University of Ostrava); Petra Beznosková (Czech Academy of Sciences, Prague); Zdeněk Paris (Institute of Parasitology, Ceske Budejovice); Leoš Shivaya Valášek (Czech Academy of Sciences, Prague); Julius Lukeš (Institute of Parasitology, Ceske Budejovice)

3B Identification of novel anti-sense regulators in *Trypanosoma brucei*

Beathrice Galili (Bar-Ilan University); K. Shanmugha Rajan (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Ron Unger (Bar-Ilan University); Christian Tschudi (Yale University); Isabel Roditi (Bern University); Shulamit Michaeli (Bar-Ilan University)

3C Role of the C- and N-terminal extensions of the *T. brucei* mRNA decapping enzyme ALPH1 in enzyme localisation, dimerization and protein interaction

Susanne Kramer (University of Würzburg); Natalia Karolak (University of Warsaw); Paula A.C. Londoño (University of Würzburg); Anna Krylova (Charles University in Prague); Johanna Odenwald (University of Würzburg); Claudia Moreira (University of Würzburg); Amelie Eden (University of Würzburg); Carina Goos (University of Würzburg); Bernardo Gabiatti (University of Würzburg); Andrea Reichert (University of Würzburg); Mark Carrington (University of Cambridge); Maria Górna (University of Warsaw); Martin Zoltner (Charles University in Prague)

3D Using metabolic labeling and scRNA-seq to understand gene expression in *Trypanosoma brucei*

Vanessa Luzak (LMU Munich); Raúl O. Cosentino (LMU Munich); Atai Dobrynin (LMU Munich); Maria Colomé-Tatché (LMU Munich); Florian Erhard (JMU Würzburg); T. Nicolai Siegel (LMU Munich)

3E deGradFP for an efficient way to degrade target proteins in *Trypanosoma brucei*

Midori Ishii (University of Oxford); Bungo Akiyoshi (University of Oxford)

TT3F Investigating trans-regulators of the *Leishmania* lifecycle

Ewan Parry (University of York); Natalia M.M. Teles (University of York); Rachel Neish (University of York); Katherine Newling (University of York); Michael J. Plevin (University of York); Angela K. Cruz (University of São Paulo, Ribeirao Preto); Jeremy C. Mottram (University of York); Pegine B. Walrad (University of York)

TT3G Genome-scale RNA interference profiling of *Trypanosoma brucei* cell cycle progression defects

Melanie Ridgway (University of Glasgow); Catarina A Marques (University of Glasgow); Michele Tinti (University of Dundee); Andrew Cassidy (University of Dundee); David Horn (University of Dundee)

TT3H Thousands of messenger RNA untranslated regions reprogram post-transcriptional gene expression profiles in trypanosomes

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

TT3I The role of KPAP1 methylation in transcript-specific mitochondrial mRNA tail addition in *Trypanosoma brucei*

Clara Smoniewski (University of Minnesota Medical School, Duluth campus); Poorya Mirzavand Borujeni (McGill University); Marshall Hampton (University of Minnesota Duluth); Reza Salavati (McGill University); Sara L Zimmer (University of Minnesota Medical School, Duluth campus)

TT3J Biogenesis of trypanosomal mitoribosomes employs conserved and lineage-specific mechanisms and multitude of assembly factors

Ondřej Gahura (Biology Centre of the Czech Academy of Sciences); Jonathan Wong (Biology Centre of the Czech Academy of Sciences); Prashant Chauhan (Biology Centre of the Czech Academy of Sciences); Shintaro Aibara (Science for Life Laboratory, Stockholm University); Vivek Singh (Science for Life Laboratory, Stockholm University); Alena Zíková (Biology Centre of the Czech Academy of Sciences); Alexey Amunts (Science for Life Laboratory, Stockholm University)

Session IV – Metabolism

Igor Cestari, Chair
Thursday 8:45 AM-11:00 AM
September 15th 2022

4A ELO1 is critical for maintenance of broad metabolic processes and efficient growth in *Trypanosoma cruzi* epimastigotes

Lucas Pagura (Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health); Peter C Dumoulin (Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health); Cameron C Ellis (Department of Biological Sciences Department, University of Texas at El Paso); Igor Estevao (Department of Biological Sciences Department, University of Texas at El Paso); Maria T Mendes (Department of Biological Sciences Department, University of Texas at El Paso); Igor C Almeida (Department of Biological Sciences Department, University of Texas at El Paso); Barbara A Burleigh (Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health)

4B Prophylactic glycan- and peptide-based vaccines elicit a protective immune response and miRNA modulation in a nonhuman primate model of Chagas disease

Priscila Silva Grijo Farani (University of Texas at El Paso); Susana Portillo (University of Texas at El Paso); Miguel A. Beltran (University of Texas at El Paso); Jacqueline Lopez (University of Texas at El Paso); Maria Tays Mendes (University of Texas at El Paso); Uriel Ortega-Rodriguez (University of Texas at El Paso); Igor Estevao (University of Texas at El Paso); Jane VandeBerg (Texas Biomedical Research Institute); Carlos R. Brito (University of Texas at El Paso); Otacilio C. Moreira (Oswaldo Cruz Institute, Oswaldo Cruz Foundation); Laura Isobel McCall (University of Oklahoma); Rosa A. Maldonado (University of Texas at El Paso); Katja Michael (University of Texas at El Paso); John L. VandeBerg (Texas Biomedical Research Institute); Igor C. Almeida (University of Texas at El Paso)

4C ROS is not just SOS: role of reactive oxygen species in metabolic remodeling during *Trypanosoma brucei* differentiation

Michaela Kunzová (University of South Bohemia, Faculty of Science); Eva Doleželová (Biology Centre CAS, Institute of Parasitology); Alena Zíková (Biology Centre CAS, Institute of Parasitology)

4D *Trypanosoma brucei* bloodstream form mitochondrion is an ATP producing organelle

Gergana Taleva (Biology Centre CAS); Michaela Husova (Biology Centre CAS); Brian Panicucci (Biology Centre CAS); Carolina Hierro Yap (Biology Centre CAS); Martin Moos (Biology Centre CAS); Petr Simek (Biology Centre CAS); Falk Butter (IMB Mainz); Frederic Bringaud (University of Bordeaux); Alena Zíková (Biology Centre CAS)

4E Tropism, immunity and pathology in the murine model of African trypanosomiasis

Chloe Barnes (Lancaster University); Nadin Fathallah (Lancaster University); John Worthington (Lancaster University); Lucy Jackson-Jones (Lancaster University); Neil Dawson (Lancaster University); Michael Urbaniak (Lancaster University)

4F Probing Fatty Acid Uptake Mechanisms in *Trypanosoma brucei*

Mufida Ammar (Clemson University); Joshua Saliutama (Clemson University); Nava Poudyal (Clemson University); Kristina Parman (Clemson University); Brianna Volbeer (Clemson University); Virginia Dellinger (Clemson University); Bailey Holder (Clemson University); Solangie Pulido-Gomez (Clemson University); Courtney Fant (Clemson University); Dan Spangler (Clemson University); Kimberly Paul (Clemson University)

TT4G A Global Perspective on the Evolutionary Impetus for Glycosomes in Kinetoplastea

Hina Durrani (University of Minnesota); Sara L Zimmer (University of Minnesota)

Session V – Gene Expression II

Pegine Walrad, Chair
Friday 8:45 AM-11:15 AM
September 16th 2022

5A Exploring the deepest secrets of the *Trypanosoma brucei* nuclear architecture

Claudia Rabuffo (LMU Munich); Anna Barcons-Simon (LMU Munich); Markus Schmidt (LMU Munich); T. Nicolai Siegel (LMU Munich)

5B Site-saturation mutagenesis of core histone tail residues in *Trypanosoma brucei*

Markéta Novotná (University of Dundee); David Horn (University of Dundee)

5C Structural Basis for Histone H3 Lysine 76 Dimethylation by *Trypanosoma brucei* DOT1A

Victoria Frisbie (Thomas Jefferson University); Hideharu Hashimoto (Thomas Jefferson University); Erik W Debler (Thomas Jefferson University)

5D The telomere binding protein UMSBP2 is a chromatin remodeler that functions in regulation of gene expression and involved in antigenic variation in trypanosomes

Awakash Soni (Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School-Hebrew University); Olga Klebanov-Akopyan (Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School-Hebrew University); Esteban Erben (Universitat Heidelberg Zentrum fur Molekulare Biologie der Universitat Heidelberg University); Amnon Harel (The Azrieli Faculty of Medicine Bar-Ilan University); Katreena Yamin (The Azrieli Faculty of Medicine Bar-Ilan University); Itay Onn (The Azrieli Faculty of Medicine Bar-Ilan University); Joseph Shlomai (Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School-Hebrew University)

5E Initiation of DNA replication in *Trypanosoma brucei* is regulated by the interaction of the RNaseH2 complex with DOT1 histone methyltransferases

Laura Hartleb (Department of Cell & Developmental Biology, Biocenter, University of Würzburg); Victoria Frisbie (Department of Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia); Hideharu Hashimoto (Department of Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia); Janna Kiselar (Department of Nutrition, School of Medicine, Case Western Reserve University, Cleveland); Falk Butter (Quantitative Proteomics, Institute of Molecular Biology (IMB), Mainz); Erik W. Debler (Department of Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia); Christian J. Janzen (Department of Cell & Developmental Biology, Biocenter, University of Würzburg)

5F RNase H1, a R-loop resolving enzyme, acts to suppress R-loop mediated DNA replication and limit genome variability in *Leishmania*

Jeziel Dener Damasceno (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Emma M. Briggs (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); João Reis-Cunha (Universidade Federal de Minas Gerais); Kathryn Crouch (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Craig Lapsley (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Daniella Bartholomeu (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Richard McCulloch (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology)

5G New Insight Into kDNA Replication through Enzymatic Characterization of POLIB

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

TT5H An orphan, kinetoplastid-specific subunit of the Origin Recognition Complex?

Catarina Marques (University of Glasgow); Marcelo da Silva (University of Sao Paulo); Jose Batista (University of Glasgow); Calvin Tiengwe (Imperial College London); Richard McCulloch (University of Glasgow)

TT5I Mosaic VSGs Form as a Consequence of DNA Damage Repair in vitro and in vivo

Jaclyn E Smith (JHUSOM); Alex Beaver (JHUSOM); Shane Gilligan-Steinberg (JHBSPH); Sarah Sudlow (JHBSPH); Monica R Mugnier (JHBSPH)

TT5J A single pseudouridine on rRNA matters: A specific effect of rRNA modification on translation in *Trypanosoma brucei*

K. Shanmugha Rajan (Bar-Ilan University); Hava Madmoni (Bar-Ilan University); Saurav Aryal (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Beathrice Galili (Bar-Ilan University); Katherina Adler (Bar-Ilan university); Ron Unger (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Shulamit Michaeli (Bar-Ilan university)

Session VI – RNA Editing

Laurie Read, Chair
Friday 2 PM-4:20 PM
September 16th 2022

6A RNA Editing: Defining Trait of Kinetoplastids

Kenneth D. Stuart (Center of Global Infectious Disease Research, Seattle Children's Research Institute)

6B Novel Roles of REH2C Helicase Complex in Editing Fidelity and as RNA-Remodeler in two life-cycle stages of *Trypanosoma brucei*

Joshua Meehan (Texas A&M University); Suzanne McDermott (Seattle Children's Research Institution); Al Ivens (University of Edinburgh); Zachary Goodall (Texas A&M University); Achim Schnaufer (University of Edinburgh); Jia Woo (Harvard Medical School); Silvi Rouskin (Harvard Medical School); Zihao Yu (Texas A&M University); Lanying Zeng (Texas A&M University); Xiren Zhang (Texas A&M University); Jorge Cruz-Reyes (Texas A&M University)

6C KREH1 RNA helicase activity promotes utilization of initiator gRNAs across multiple mRNAs in trypanosome RNA editing

Ashutosh P Dubey (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Brianna L Tylec (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Amartya Mishra (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Runpu Chen (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Yijun Sun (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Laurie K Read (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo)

6D Structure of RNA Editing Substrate Binding Complex

Ruslan Afasizhev (Boston University); Shiheng Liu (UCLA); Inna Afasizheva (Boston University); Hong Wang (Boston University); Ana Lucia Alvarez-Cabrera (UCLA); Xiaorun Li (UCLA); Jane Lee (UCLA); Jason Hu (UCLA); Zihang Li (UCLA); Clinton Yu (UC Irvine); Lan Huang (UC Irvine); Fan Zhang (Shanghai Tech); Liye Zhang (Shanghai Tech); Z. Hong Zhou (UCLA)

6E Domain function and structure of three heterodimeric endonuclease subunits in RNA editing catalytic complexes

Suzanne McDermott (Seattle Childrens Research Institute); Jason Carnes (Seattle Childrens Research Institute); Isaac Lewis (Seattle Childrens Research Institute); Maxwell Tracy (Seattle Childrens Research Institute); Ken Stuart (Seattle Childrens Research Institute)

6F TUTase-Exonuclease-RNA Helicase Nexus Governs Mitochondrial RNA Biogenesis in Trypanosomes

Inna Afasizheva (Boston University); Takuma Suematsu (Boston University); Andres Vacas (Boston University); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine)

Session VII – Antigenic Variation

Christian Janzen, Chair
Saturday 9 AM-10:30 AM
September 17th 2022

7A Extravascular spaces are reservoirs of antigenic diversity in *Trypanosoma brucei*

Alexander Beaver (Johns Hopkins School of Medicine); Nathan P Crilly (Johns Hopkins School of Medicine); Bailin Zhang (Johns Hopkins Bloomberg School of Public Health); Bryce Bobb (Johns Hopkins Bloomberg School of Public Health); Filipa Rijo-Ferreira (Berkeley Public Health Molecular and Cell Biology Department); Luísa M Figueiredo (Universidade de Lisboa); Monica Mugnier (Johns Hopkins Bloomberg School of Public Health)

7B Allosteric regulation of repressor-activator protein 1 by PI(3,4,5)P3 controls on and off switching of telomeric expression sites in trypanosomes

Abdoulie O. Touray (McGill University); Rishi Rajesh (McGill University); Tony Isebe (McGill University); Mira Loock (McGill University); Oksana Kutova (McGill University); Igor Cestari (McGill University)

7C Multi-allelic exclusion by an allele-selective RNA-DNA helicase in African trypanosomes

Joana RC Faria (York Biomedical Research Institute, Department of Biology, University of York, UK); Hideharu Hashimoto (Thomas Jefferson University, USA); Michele Tinti (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, UK); Catarina A Marques (Wellcome Trust Centre for Integrative Parasitology, University of Glasgow, UK); Erik W Debler (Thomas Jefferson University, USA); David Horn (University of Dundee, UK)

7D Heterogeneous elongation of RNA polymerase I transcription at the active VSG expression site in *Trypanosoma brucei*

James Budzak (Imperial College London); Gloria Rudenko (Imperial College London)

7E The RNA Recognition Motif (RRM)-mediated RNA binding activity in *T. brucei* RAP1 is essential for VSG monoallelic expression

Amit Kumar Gaurav (Cleveland State University); Marjia Afrin (Cleveland State University); Xian Yang (The Hong Kong Polytechnic University); Arpita Saha (Cleveland State University); Xuehua Pan (The Hong Kong Polytechnic University); Zeyang Ji (Hong Kong University of Science and Technology); Kam-Bo Wang (The Chinese University of Hong Kong); Mingjie Zhang (Hong Kong University of Science and Technology); Yanxiang Zhao (The Hong Kong Polytechnic University); Bibo Li (Cleveland State University)

7F *Trypanosoma brucei* RNA/DNA hybrid interactomes reveal a role for RAD51 in R-loop homeostasis and repair of VSG-localised DNA breaks during antigenic variation

Mark Girasol (University of Glasgow); Emma Briggs (University of Edinburgh); Jeziel Damasceno (University of Glasgow); Catarina Marques (University of Glasgow); Marija Krasilnikova (University of Glasgow); Craig Lapsley (University of Glasgow); Ross Carruthers (University of Glasgow); Dario Beraldi (University of Glasgow); Pilarita Rivera (University of the Philippines); Richard McCulloch (University of Glasgow)

Poster Session A

Wednesday 7 PM
September 14th 2022

1 TT1G The role of ESCRT in endocytosis and secretion of extracellular vesicles trypanosomatids

Nadjania S de L Silva (Unifesp); Suellen R Maran (Unifesp); Nilmar S Moretti (Unifesp); Ana C Torrecilhas (Unifesp); Sergio Schenkman (Unifesp)

2 TT1H Spatial regulation of the multifunctional dynamin-1-like protein in *Trypanosoma brucei*

Madeline F Malfara (Villanova University); Alexa Pereira (Villanova University); Megan L Povelones (Villanova University)

3 TT2E A quantitative mass spectrometry approach to identify novel proteins involved in macrophage infections by *Leishmania* parasites

Nicolas Hagedorn (Department of Cell and Developmental Biology, Würzburg, Germany); Albert Fradera Sola (Institue of Molecular Biology, Mainz); Elisa Theres Rauh (Department of Cell and Developmental Biology, Würzburg, Germany); Tobias Gold (Microbiology Institute-Clinical Microbiology, Erlangen); Ulrike Schleicher (Microbiology Institute-Clinical Microbiology, Erlangen); Falk Butter (Institue of Molecular Biology, Mainz); Christian J. Janzen (Department of Cell and Developmental Biology, Würzburg, Germany)

4 A human adipose tissue model for *Trypanosoma brucei* infection

Laura Hauf (Universität Würzburg); Carina Blum (Universitätsklinikum Würzburg); Torsten Blunk (Universitätsklinikum Würzburg); Markus Engstler (Universität Würzburg)

5 TT2F Trypanosome sequestration and immune response drive acute cerebral disease

Sara Silva Pereira (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Mariana De Niz (Institut Pasteur); Karine Serre (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Joana Coelho (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Marie Ouarné (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa "); Cláudio A Franco (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

6 TT2G Development of a molecular diagnostic toolbox for the detection of trypanosomatid parasites by SHERLOCK

Elena Pérez-Antón (Institut Pasteur, Université Paris Cité); Roger Junior Eloiflin (Institut

de Recherche pour le Développement (IRD)-CIRAD); Annick Dujeancourt-Henry (Institut Pasteur, Université Paris Cité); Aïssata Camara (Institut Pasteur of Guinea); Jean-Mathieu Bart (Institut de Recherche pour le Développement (IRD)-CIRAD); Brice Rotureau (Institut Pasteur, Université Paris Sorbonne; and Institut Pasteur of Guinea); Lucy Glover (Institut Pasteur, Université Paris Cité)

7 TT2H Fexinidazole survival genes implicate novel redox pathway in drug resistance

Arthur Drouaud (George Washington University); Jenna Murrow (George Washington University); Galadriel Hovel-Miner (George Washington University)

8 TT2I A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease

Tamara Sternlieb (Institute of Parasitology, McGill University); Rhiannon Heslop (Institute of Parasitology, McGill University); Mira Loock (Institute of Parasitology, McGill University); Luisa Berenguer Antunes (Institute of Parasitology, McGill University); Igor Cestari (Institute of Parasitology, McGill University;Division of Experimental Medicine, McGill University)

9 TT2J Structure-guided discovery of a small molecule inhibitor targeting *Trypanosoma brucei* Replication Protein A1

Aditi Mukherjee (Rutgers University, PHRI); Zakir Hossain (Queens College); Junyong Choi (CUNY Graduate Center, Queens College); Hee-Sook Kim (Rutgers University, PHRI)

10 TT3F Investigating trans-regulators of the *Leishmania* lifecycle

Ewan Parry (University of York); Natalia M.M. Teles (University of York); Rachel Neish (University of York); Katherine Newling (University of York); Michael J. Plevin (University of York); Angela K. Cruz (University of São Paulo, Ribeirao Preto); Jeremy C. Mottram (University of York); Pegine B. Walrad (University of York)

11 TT3G Genome-scale RNA interference profiling of *Trypanosoma brucei* cell cycle progression defects

Melanie Ridgway (University of Glasgow); Catarina A Marques (University of Glasgow); Michele Tinti (University of Dundee); Andrew Cassidy (University of Dundee); David Horn (University of Dundee)

12 TT3H Thousands of messenger RNA untranslated regions reprogram post-transcriptional gene expression profiles in trypanosomes

Anna Trenaman (University of Dundee); Michele Tinti (University of Dundee); Richard

Wall (University of Dundee); David Horn (University of Dundee)

13 TT3I The role of KPAP1 methylation in transcript-specific mitochondrial mRNA tail addition in *Trypanosoma brucei*

Clara Smoniewski (University of Minnesota Medical School, Duluth campus); Poorya Mirzavand Borujeni (McGill University); Marshall Hampton (University of Minnesota Duluth); Reza Salavati (McGill University); Sara L Zimmer (University of Minnesota Medical School, Duluth campus)

14 TT3J Biogenesis of trypanosomal mitoribosomes employs conserved and lineage-specific mechanisms and multitude of assembly factors

Ondřej Gahura (Biology Centre of the Czech Academy of Sciences); Jonathan Wong (Biology Centre of the Czech Academy of Sciences); Prashant Chauhan (Biology Centre of the Czech Academy of Sciences); Shintaro Aibara (Science for Life Laboratory, Stockholm University); Vivek Singh (Science for Life Laboratory, Stockholm University); Alena Zíková (Biology Centre of the Czech Academy of Sciences); Alexey Amunts (Science for Life Laboratory, Stockholm University)

15 Amino acid uptake in *Leishmania* virulence

Jamal Asseri (University of Glasgow); Richard Burchmore (University of Glasgow); Harry De-koning (University of Glasgow)

16 Cracking the vault

Anna Zavřelová (Faculty of Science, Charles University in Prague); Siqi Shen (Faculty of Science, Charles University in Prague); Martin Zoltner (Faculty of Science, Charles University in Prague)

17 Probing the role of regulation loss in compartmentalized phosphofructokinase in trypanosomatids

Austin Petersen (University of Minnesota Duluth); Jon Rumbley (University of Minnesota College of Pharmacy, Duluth Campus); Sara L Zimmer (University of Minnesota Medical School, Duluth Campus)

18 Expanding the unusual mRNA export platform of Trypanosomes

Bernardo Papini Gabiatti (University of Würzburg); Silke Braune (University of Würzburg); Timothy Krüger (University of Würzburg); Johanna Odenwald (University of Würzburg); Susanne Kramer (University of Würzburg)

19 The molecular basis of heat shock signalling in African trypanosomes

Caroline Dewar (Lancaster University); Marianne Aelmans (Lancaster University); Mick Urbaniak (Lancaster University)

20 Genetic deletion of pyrroline-5-carboxilate synthetase and pyrroline-5-carboxylate reductase suggests a shortcut in the glutamate-proline pathway and the direct use of glutamate as energy source in *Leishmania braziliensis*

Gustavo Daniel Campagnaro (Ribeirão Preto Medical School - USP); Angela Kaysel Cruz (Ribeirão Preto Medical School - USP)

Poster Session B

Thursday 7 PM
September 15th 2022

21 Synthetic glycovaccines elicit cross-protection against Chagas disease and cutaneous Leishmaniasis in a murine co-infection model.

Colin D Knight (University of Texas at El Paso); Igor Estevao (University of Texas at El Paso); Susana Portillo (University of Texas at El Paso); Priscila Farani (University of Texas at El Paso); Alba Montoya (University of Texas at El Paso); Elisa Carvajal (University of Texas at El Paso); Irodiel Vinales (University of Texas at El Paso); Felipe Rodriguez (University of Texas at El Paso); Rosa A. Maldonado (University of Texas at El Paso); Katja Michael (University of Texas at El Paso); Igor C. Almeida (University of Texas at El Paso)

22 Cytokinesis in *Trypanosoma brucei* relies on an orphan kinesin that dynamically crosslinks microtubules

Thomas E Sladewski (University of Connecticut); Paul C Campbell (Brown University); Neil Billington (NHLBI); Alexandra D'Ordine (Brown University); Christopher L de Graffenried (Brown University)

23 On the trail of the new jobs of TbPam18 and TbPam16

Corinne von Känel (Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern); Silke Oeljeklaus (Biochemistry and Functional Proteomics, Institute of Biology II, Faculty of Biology and Signalling Research Centres BIOSS and CIBSS, University of Freiburg); Salvatore Calderaro (Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern); Bettina Warscheid (Biochemistry and Functional Proteomics, Institute of Biology II, Faculty of Biology and Signalling Research Centres BIOSS and CIBSS, University of Freiburg); André Schneider (Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern)

24 Single cell transcriptomic analysis of the *T. brucei* cell cycle and differentiation deciphers cell cycle progression and exit

Emma Marie Briggs (University of Edinburgh); Catarina Marques (University of Glasgow); Thomas Dan Otto (University of Glasgow); Keith R Matthews (University of Edinburgh)

25 The histone methyltransferase DOT1B is dispensable for stage differentiation in *Leishmania mexicana* but not for efficient infection of macrophages

Elisa Theres Rauh (Department of Cell & Developmental Biology, Biocenter, University of Würzburg); Nicole Eisenhuth (Department of Cell & Developmental Biology, Biocenter, University of Würzburg); Falk Butter (Quantitative Proteomics, Institute of Molecular Biology (IMB), Mainz); Andrea Debus (Microbiology Institute-Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-

Alexander-University, Erlangen); Ulrike Schleicher (Microbiology Institute-Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander-University, Erlangen); Christian J. Janzen (Department of Cell & Developmental Biology, Biocenter, University of Würzburg)

26 Exploring the cellular targets of anti-Leishmanial natural product analogues using fluorescence microscopy

Hannah A Asiki (University of Oxford); Richard J Wheeler (University of Oxford); Edward A Anderson (University of Oxford)

27 TT4G A Global Perspective on the Evolutionary Impetus for Glycosomes in Kinetoplastea

Hina Durrani (University of Minnesota); Sara L Zimmer (University of Minnesota)

28 KRGG1 is essential and required for normal editing in bloodstream form *Trypanosoma brucei*

Jason Carnes (Seattle Children's Research Institute); Claire Gendrin (Seattle Children's Research Institute); Suzanne M. McDermott (Seattle Children's Research Institute); Ken Stuart (Seattle Children's Research Institute)

29 Does *Leishmania* ATM play a role in DNA repair and genome plasticity?

Jennifer A Black (University of Sao Paulo); Gabriel L A Silva (University of Glasgow); Luiz R O Tosi (University of Sao Paulo)

30 Investigating parasite diversity during congenital transmission of *Trypanosoma cruzi*

Jill Hakim (Johns Hopkins); Freddy Tinajeros (Asociación Benéfica PRISMA); Andreea Waltmann (University of North Carolina at Chapel Hill); Oksana Kharabora (University of North Carolina at Chapel Hill); Edith Malaga Machaca (Universidad Peruana Cayetano Heredia); Manuela Verastegui (Universidad Peruana Cayetano Heredia); Maria Carmen Meduiña (Hospital Percy Boland Rodríguez); John Juliano (University of North Carolina at Chapel Hill); Robert Gilman (Johns Hopkins); Monica Mugnier (Johns Hopkins); Natalie Bowman (University of North Carolina at Chapel Hill); Working Group on Chagas Disease in Bolivia and Peru

31 Automation and optimization of nucleotide extraction method to improve detection of *Trypanosoma cruzi* satellite DNA in the blood of chronically infected patients

Jacqueline Lopez (University of Texas at El Paso); Priscila S. G Farani (University of Texas at El Paso); Otacilio C Moreira (Oswaldo Cruz Institute (Fiocruz)); Igor C Almeida

(University of Texas at El Paso)

32 Trapped in Export: NUP-mRNA interactions in trypanosomes

Johanna Odenwald (Julius-Maximilians-Universität Würzburg); Siqi Shen (Charles University Prague); Bernardo Gabiatti (Julius-Maximilians-Universität Würzburg); Claudia Moreira (Carlos Chagas Institute Curitiba); Martin Zoltner (Charles University Prague); Susanne Kramer (Julius-Maximilians-Universität Würzburg)

33 Overexpression of RNA Editing Helicase 2 (KREH2) Wild-Type and Dominant Negative Disrupts Editing in African Trypanosomes

Joshua Meehan (Texas A&M University); Suzanne McDermott (Seattle Children's Research Institution); Al Ivens (University of Edinburgh); Achim Schnaufer (University of Edinburgh); Sunil Kumar Sharma (Texas A&M University); Zachary Goodall (Texas A&M University); Xiuren Zhang (Texas A&M University); Jorge Cruz-Reyes (Texas A&M University)

34 Massive Upregulation of COII mRNA Editing Correlates with Alterations in RESC Protein and RNA Interactions During *T. brucei* Differentiation

Joseph T. Smith Jr. (University at Buffalo); Arunasalam Naguleswaran (University of Bern); Brianna Tylec (University at Buffalo); Isabel Roditi (University of Bern); Laurie K. Read (University at Buffalo)

35 Visualizing the process of variant surface glycoprotein shedding in response to trypanosome lytic factor

Kayla Leiss (Hunter College department of biological sciences); Alisha Rancho-Jensen (Hunter College department of biological sciences); Sophio Kirimlishvili (Hunter College department of biological sciences); Sara Fresard (Hunter College department of biological sciences); Jayne Raper (Hunter College department of biological sciences)

36 Glycosomal Protein kinase A in *T. brucei*: a potential regulator of metabolism?

Kristina Malenica (Faculty of Biology, Ludwig-Maximilians-University Munich); Ignasi Forne (Biomedical Center, Ludwig-Maximilians-University Munich); Axel Imhof (Biomedical Center, Ludwig-Maximilians-University Munich); Frederic Bringaud (MFP (CNRS UMR-5234), University of Bordeaux); Michael Boshart (Faculty of Biology, Ludwig-Maximilians-University Munich)

37 Assessing Antigenic Distinction of Novel Mosaic VSGs found in *Trypanosoma brucei*

Kevin J Wang (JHSPH); Jaclyn Smith (JHSPH); Monica Mugnier (JHSPH)

38 *T. brucei* heterogeneity in the adipose tissue

Lara Lopez Escobar (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Sandra Trindade (Instituto de Medicina Molecular João Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Milena Jakimovska Ozdemir (Acidabem University); Ugur Sezerman (Acidabem University); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

39 Phenotypic and genotypic characterisation of *Leishmania* (Viannia) *braziliensis* clinical isolates with variable susceptibility to AmB.

Laura Gonzalez (Universidad de los Andes); Carolina Vargas (Universidad Nacional de Colombia - Sede Bogotá); Samanda Aponte (Universidad Nacional de Colombia - Sede Bogotá); Aura Rodriguez (universidad del Tolima); Liliana Bonilla (Universidad Nacional de Colombia - Sede Bogotá); Marcela Parra-Muñoz (Universidad Nacional de Colombia - Sede Bogotá); Juan Matiz (Universidad Nacional de Colombia - Sede Bogotá); Maria P. Rodirguez (Universidad Nacional de Colombia - Sede Bogotá); Daniel Urrea (Universidad del Tolima); Joge Duitama (Universidad de los Andes); Maria Echeverry (Universidad Nacional de Colombia - Sede Bogotá)

40 Identification of *Trypanosoma cruzi* flagellar proteins in replicative stages

Madalyn M Won (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health); Barbara A Burleigh (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health)

Poster Session C

Friday 7 PM
September 16th 2022

41 TT5H An orphan, kinetoplastid-specific subunit of the Origin Recognition Complex?

Catarina Marques (University of Glasgow); Marcelo da Silva (University of Sao Paulo); Jose Batista (University of Glasgow); Calvin Tiengwe (Imperial College London); Richard McCulloch (University of Glasgow)

42 TT5I Mosaic VSGs Form as a Consequence of DNA Damage Repair in vitro and in vivo

Jaclyn E Smith (JHUSOM); Alex Beaver (JHUSOM); Shane Gilligan-Steinberg (JHBSPH); Sarah Sudlow (JHBSPH); Monica R Mugnier (JHBSPH)

43 TT5J A single pseudouridine on rRNA matters: A specific effect of rRNA modification on translation in *Trypanosoma brucei*

K. Shanmugha Rajan (Bar-Ilan University); Hava Madmoni (Bar-Ilan University); Saurav Aryal (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Beathrice Galili (Bar-Ilan University); Katherina Adler (Bar-Ilan university); Ron Unger (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Shulamit Michaeli (Bar-Ilan university)

44 Using yeast surface display to identify phosphoinositide-binding proteins in trypanosomes

Mengjin Gao (McGill University); Andressa Brito Lira (McGill University); Tamara Sternlieb (McGill University); Mira Loock (McGill University); Igor Cestari (McGill University)

45 Cleavage of *Trypanosoma brucei* FoF1-ATP synthase alpha subunit: A rare attribute

Minal Jain (Institute of Parasitology, Biology Centre, CAS and University of South Bohemia); Karolina Subrtova (School of Biological Sciences, University of Edinburgh); Carolina Hierro Yap (Institute of Parasitology, Biology Centre, CAS); Brian Panicucci (Institute of Parasitology, Biology Centre, CAS); Achim Schnaufer (School of Biological Sciences, University of Edinburgh); Alena Zíková (Institute of Parasitology, Biology Centre, CAS and University of South Bohemia)

46 Heme Homeostasis at the *Leishmania*-Host Interface

Melissa Perry (University of Maryland); Hui-wang Ai (University of Virginia); Phil Yates (Oregon Health & Science University); Iqbal Hamza (University of Maryland)

47 Single-cell RNA-sequencing: When things go wrong…

T. Nicolai Siegel (LMU Munich); Kirsty R McWilliam (University of Edinburgh); Raúl O

Cosentino (LMU Munich); Zhibek Keneskhanova (LMU Munich); Atai Dobrynin (LMU Munich); Maria Colomé-Tatché (LMU Munich)

48 Visualizing metacyclic VSG coat synthesis in live trypanosomes

Nikolay G Kolev (Yale School of Public Health); Paul C Campbell (Brown University); Huafang Shi (Yale School of Public Health); Christopher L de Graffenried (Brown University); Christian Tschudi (Yale School of Public Health)

49 TriTrypDB: Free online informatic tools for kinetoplastid biologists

Omar S. Harb (University of Pennsylvania); David Starns (University of Liverpool); David S. Roos (University of Pennsylvania); on behalf of the VEuPathDB project.

50 Establishing a CRISPR-dCas visualization system to study genome architecture changes in *T. brucei*

Prateek Yadav (LMU Munich); Richard Wheeler (University of Oxford); T. Nicolai Siegel (LMU Munich)

51 A branched chain amino acids transporter in the procyclic form of *Trypanosoma brucei*

Quentin Oliveres (Institute of Plant Sciences); Juan P. Macêdo (Institute of Plant Sciences); Silvan Piller (Institute of Plant Sciences); Alexander Haindrich (Institute of Plant Sciences); Doris Rentsch (Institute of Plant Sciences)

52 From TrypTag to LeishTag: Conservation of localisation?

Karen Billington (Oxford Brookes University); Ulrich Dobramysl (University of Oxford); Jeremy Mottram (University of York); Eva Gluenz (University of Glasgow); Jack D Sunter (Oxford Brookes University); Richard J Wheeler (University of Oxford)

53 Identification of RNA binding targets and protein interactors of the *Trypanosoma brucei* RNA-binding protein 7B during metacyclogenesis

Saúl Rojas Sánchez (Yale School of Public Health); Christian Tschudi (Yale School of Public Health)

54 TLF3: Identification of a unique pre-beta high density lipoprotein that contributes to immunity against African Trypanosomes

Sara Fresard (Hunter College and The Graduate Center, City University of New York); Russell Thomson (Hunter College, City University of New York); Sophio Kirimlishvili (Hunter College, City University of New York); Jayne Raper (Hunter College and The Graduate Center, City University of New York)

55 APEX2 proximity labelling for mapping mRNP-NUP interactions during nuclear export

Siqi Shen (Charles University); Johanna Odenwald (University of Würzburg); Bernardo Gabiatti (University of Würzburg); Susanne Kramer (University of Würzburg); Martin Zoltner (Charles University)

56 *Trypanosoma brucei* TEL2 suppresses VSG switching

SK Abdus Sayeed (Cleveland State University); Bibo Li (Cleveland State University)

57 *Trypanosoma carassii*, a model for whole host interaction studies.

Sarah Gaëlle Monic (University of Cambridge , Biochemistry department); Maria Forlenza (Wageningen University and Research); Mark Carrington (University of Cambridge, Biochemistry department)

58 Track & trace: using dual-colour imaging to visualise transport in the beating flagella of *Leishmania mexicana*

Sophie Gray (University of Oxford); Richard Wheeler (University of Oxford)

59 RESC8 and RESC14 cooperate to mediate RESC function and dynamics during trypanosome RNA editing

Katherine Sortino (University at Buffalo, SUNY); Brianna Tylec (University at Buffalo, SUNY); Natialie M McAdams (University at Buffalo, SUNY); Runpu Chen (University at Buffalo, SUNY); Yijun Sun (University at Buffalo, SUNY); Laurie K Read (University at Buffalo, SUNY)

60 Identifying telomeric expression site protein interactions at peptide resolution using cross-linking and mass spectrometry

Tony Isebe (McGill University); Igor Cestari (McGill University)

61 High-throughput scRNA-seq in *Trypanosoma brucei* with Smart-seq3express

Zhibek Keneskhanova (Ludwig-Maximilian University Munich); Kirsty McWilliam (The University of Edinburgh); Raúl Cosentino (Ludwig-Maximilian University Munich); T. Nicolai Siegel (Ludwig-Maximilian University Munich)

ABSTRACTS

Session I Abstracts – Cell Biology

Christopher de Graffenried, Chair
Tuesday 7-9 PM
September 13th 2022

1A The *Trypanosoma cruzi* amastigote flagellum is motile and displays aperiodic beating

Madalyn M Won (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health); Timothy Krüger (Department of Cell- and Developmental Biology, Biozentrum, University of Würzburg); Markus Engstler (Department of Cell- and Developmental Biology, Biozentrum, University of Würzburg); Barbara A Burleigh (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health)

Over its complex life cycle, *Trypanosoma cruzi* adapts to disparate host environments by transitioning between elongated motile extracellular forms (epimastigote, trypomastigote) and rounded non-motile intracellular forms (amastigote). In mammals, motile trypomastigotes invade host cells and quickly transition to amastigotes that replicate in the host cell cytoplasm. Accompanying the loss of motility in the intracellular amastigote stage is a dramatic shortening of the flagellum, which is often referred to as a 'remnant' and assumed to be functionally inert. Here, we report for the first time that the *T. cruzi* amastigote flagellum is motile and undergoes aperiodic beating in the host cell cytosol. Analyzing high-resolution kymographs, we have precisely measured the oscillatory motility and determined an average beat frequency of ~1 Hz, or 1 beat per second for the amastigote flagellum, with consistent results obtained for both intracellular and externalized parasites. Amastigote flagellar beating was observed in four different *T. cruzi* strains and in both human and mouse-derived host cells. Exposure of amastigotes to a potent parasite mitochondrial electron transport inhibitor, GNF7876, resulted in marked inhibition of flagellar beating which is restored following compound washout. These data indicate that mitochondrially-generated ATP is required to fuel flagellar movement in *T. cruzi* amastigotes. Further studies are underway to assess the roles of calcium and cAMP as potential regulators of *T. cruzi* amastigote flagellar beat. Thus, these novel observations dramatically alter our view of the *T. cruzi* amastigote flagellum and point to the potential of this organelle to play an active role in the infection process.

1B cAMP signaling controls adhesion of *Crithidia fasciculata*

Nikki A Holmes (Villanova University); Kelly R Hodges (Villanova University); Madeline F Malfara (Villanova University); Megan L Povelones (Villanova University)

Crithidia fasciculata is a mosquito-infectious kinetoplastid parasite. All kinetoplastids adhere to tissues in their insect hosts via the flagellum. While the mechanism is unknown, *C. fasciculata* serves as a useful model for this process because of its ability to adhere in vitro. cAMP is a second messenger involved in many kinetoplastid processes, including differentiation, social motility, and flagellar movement, but the pathway is uncharacterized in C. fasciculata. Two important enzymes in the cAMP pathway are adenylyl cyclases (ACs) and phosphodiesterases (PDEs), which create and degrade cAMP respectively. We have performed transcriptomic and proteomic analyses comparing swimming and adhered C. fasciculata, showing that cAMP signaling components, including both PDEs and ACs, are upregulated in the swimming stage. We have localized both a putative phosphodiesterase (CfPDEA) and a putative adenylyl cyclase (CfRAC1) to the flagellum. The PDEA is found along the length of the flagellum, while the RAC1 is primarily found in the distal third of the flagellum. Other kinetoplastids have displayed this same localization pattern, further supporting the flagellum as a signaling organelle. During adherence, *C. fasciculata* shortens the flagellum and undergoes multiple cell divisions as adhered haptomonads, forming rosettes. When a single allele of the phosphodiesterase PDEB1 is knocked out, *C. fasciculata* parasites initially adhere but are delayed in the morphological transition to adherence, which includes both flagellar shortening and cell rounding. Treatment with phosphodiesterase inhibitors intensifies this effect. Understanding the cAMP pathway in *C. fasciculata* may reveal the role of the flagellum in signal transduction and adherence in kinetoplastids.

1C Kinetoplastid Attachment Proteins (KAPs): Functional dissection of the *Leishmania* - sand fly attachment interface

Ryuji Yanase (Oxford Brookes University); Flavia Moreira-Leite (Oxford Brookes University); Edward Rea (Oxford Brookes University); Katerina Pruzinova (Charles University); Jovana Sádlová (Charles University); Petr Volf (Charles University); Jack Sunter (Oxford Brookes University)

Within the sand fly vector, *Leishmania* parasites have two major morphological forms, a motile promastigote and a haptomonad, which is attached to the stomodeal valve through a shortened and modified flagellum. Dissecting haptomonad development and attachment is critical to understanding parasite transmission; however, studies of haptomonads are limited, as this is a technically challenging life cycle form to investigate. To gain an in-depth understanding of the in vivo haptomonad cellular architecture and organisation, we combined two volume electron microscopy techniques – serial block face-scanning electron microscopy (SBF-SEM) and serial electron microscopy tomography – generating high resolution 3D models of haptomonads attached to the stomodeal valve. Haptomonads were densely packed around the valve and were attached through the tip of a shortened flagellum. The attachment interface was filled, on the flagellum side, with an electron-dense plaque that connected to abundant filaments and filament bundles. Next, we generated attached L. mexicana haptomonads in vitro and confirmed that the fine ultrastructure of these forms was comparable to that of haptomonads found in vivo. Using comparative proteomic approaches, we identified proteins locating to the attachment interface and a number of these proteins are present in other kinetoplastid parasites. Deletion analysis using CRISPR/Cas9 compromised *Leishmania* attachment both in vitro and in the sand fly, confirming that we have identified critical components of the attachment interface. This provides the first molecular insights into a kinetoplastid parasite vector attachment interface, which will underpin our understanding of this crucial interaction.

1D Providing stability without being rigid – the dual nature of the TAC in *Trypanosoma brucei*

Irina Bregy (University of Bern); Julika Radecke (Diamond Light Source); Akira Noga (Paul Scherrer Institute); Mara Kern (University of Bern); Beat Haenni (University of Bern); Takashi Ishikawa (Paul Scherrer Institute); Alistair Siebert (Diamond Light Source); Benoît Zuber (University of Bern); Torsten Ochsenreiter (University of Bern)

In contrast to most eukaryotic cells, trypanosomes only contain a single mitochondrion per cell. Within that singular mitochondrion, the protist carries a single mitochondrial DNA network (kDNA) that is arranged as a disc of interlocked DNA circles. Segregation of the replicated kDNA network is organized by the basal body of the singular flagellum. At the proximal end of the basal body, trypanosomes have a structure termed the Tripartite Attachment Complex (TAC). The TAC physically connects the basal body and the pro-basal body to the kDNA. This allows for anchoring of the kDNA throughout the cell cycle, couples kDNA segregation with the separation of the basal bodies prior to cell division. While many components of the TAC have been identified, very little was known about the structure of the complex. To shed light on the TAC structure, we generated a population of ultra-thin trypanosomes (as demonstrated by Sun et al., 2018) and use those cells to perform cryo-electron tomography (cryo-ET). Using cryo-ET, we are able to acquire three-dimensional high resolution imagery of the TAC. This allows us to appreciate how the TAC offers great mechanical flexibility for basal body movement. We are able to measure the dimensions of individual protein components and sub-compartments of the TAC, as well as the overall orientation and positioning of the basal bodies towards the mitochondrial pocket. Using the collective information from individual proteins and protein complexes together with the data on orientation and positioning of the basal bodies, we are able to describe the TAC in high detail. The findings clearly demonstrate how the TAC is a stable connector of the basal body and the pro-basal body to the kDNA, while keeping the system flexible for extensive repositioning events of the basal bodies and the mechanical forces involved in kDNA segregation.

1E Structural studies of the ciliary component CFAP410 reveal a bimodular protein and explain how its mutations cause ciliopathies

Alexander Stadler (Medical Univeristy of Vienna); Heloise Gabriel (Oxford Brookes University); Sue Vaughan (Oxford Brookes University); Jack Sunter (Oxford Brookes University); Gang Dong (Medical University of Vienna)

Cilia and flagella are antenna-like organelles protruding from most cells in the human body. Defects in ciliary structure or function often lead to human diseases that are collectively called ciliopathies. The cilia and flagella associated protein 410 (CFAP410) localizes at the basal body of the cilium and plays essential roles in both ciliogenesis and DNA double-strand break (DSB) repair. Multiple single amino acid mutations in CFAP410 have been identified in patients, which led to two distinct ciliopathies: retinal dystrophy with or without macular staphyloma (RDMS) and spondylometaphyseal dysplasia, axial (SMDAX). However, the molecular mechanism for how the mutations in CFAP410 cause these disorders remains unknown due to a lack of any high-resolution structure of the protein. CFAP410 is highly conserved in many organisms from algae to humans. We have carried out extensive investigations on *Trypanosoma brucei* CFAP410, as well as its homologs in humans and *Chlamydomonas reinhardtii*. Our work culminated multiple high-resolution crystal structures of these proteins, which reveal that CFAP410 is a bimodular protein comprising two distinct domains: a leucine rich repeat (LRR) motif at the N-terminus and an oligomeric helical bundle at the C-terminus. These two domains are interconnected via a long unstructured loop. We can also clearly view the locations of the disease-causing mutation sites in our crystal structures. Taken together with our in vivo localization data from *T. brucei*, we could now provide an explanation how the mutations in CFAP410 cause the aforementioned retinal and skeletal ciliopathies.

1F A cocktail of VSG-related antigens and digestive enzymes is quantitatively enriched in the African trypanosome secretome

Gustavo Bravo Ruiz (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); Jordan Alexander Forteath (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); Michele Tinti (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); Shravya Murali (The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee); David Horn (University of Dundee)

Variant surface glycoproteins (VSGs) coat parasitic African trypanosomes and underpin antigenic variation and immune evasion by periodically switching the expressed VSG. For this, trypanosomes have a vast repertoire of VSG genes. There is also a subset of VSG-related (VR) genes which differ from other VSG-like genes in expression patterns and genomic location. In this study, we use a range of bioinformatics tools to undertake a detailed analysis of *Trypanosoma brucei* VRs, finding more than 30 chromosome-internally located VR genes. VRs are predicted to be structurally similar to VSGs, incorporating putative signal peptides but with little support for GPI-anchor signals, suggesting that they may be secreted. Using quantitative proteomics and transcriptomics, we find that VRs are expressed in a bloodstream-form specific manner, and secreted together with other VSG-like proteins. We also show that VRs are conserved in the human-infective species *T. brucei* gambiense, while few VRs were identified in *Trypanosoma congolense*. Co-secretion of VRs and a cocktail of digestive enzymes by *T. brucei*, suggests a role in modulating the host immune response. We will further monitor the fate of these secreted proteins by expressing VSGs and VRs with specific epitope-tags.

TT1G The role of ESCRT in endocytosis and secretion of extracellular vesicles trypanosomatids

Nadjania S de L Silva (Unifesp); Suellen R Maran (Unifesp); Nilmar S Moretti (Unifesp); Ana C Torrecilhas (Unifesp); Sergio Schenkman (Unifesp)

The ESCRT machinery, consists of four multi-subunit complexes, ESCRT-0 to IV involved in the transport of cargoes to form multivesicular bodies in cell endosomal compartments. These endosomes fuse with lysosomes for cargo degradation or with the plasma membrane to produce extracellular vesicles. Here, we studied the role of the ESCRT-I complex in *Trypanosoma cruzi* and *Leishmania mexicana*. Parasites expressing Cas9 and T7RNA polymerase were transfected with DNA fragments coding for two sgRNA to cleave the coding sequence ends of the VPS23 gene, a member of the ESCRT-I, together with DNA donors coding for hygromycin and/or blasticidin resistance. Only one of the VPS23 alleles could be replaced, while several other double knockouts were obtained, suggesting that VPS23 is essential for *T. cruzi* and *L. mexicana*. Nevertheless, these partial knockouts significantly lower the receptor mediated endocytosis of transferrin, but not the fluid phase BSA-uptake in *T. cruzi*. In addition, they reduced the secretion of extracellular vesicles by *T. cruzi* epimastigotes. The partial knockout did not affect proliferation and metacyclogenesis, and the resulting *T. cruzi* trypomastigotes were able to infect cells as the parental cell lines. We are currently analyzing whether the secretion of extracellular vesicles is also changed in *T. cruzi* trypomastigotes released from infected mammalian cells and the capacity of these parasites to promote infection. These results suggested an essential role of TcVPS23 by modulating endocytic and secretory activities in both *T. cruzi* and *L. mexicana*.

TT1H Spatial regulation of the multifunctional dynamin-1-like protein in *Trypanosoma brucei*

Madeline F Malfara (Villanova University); Alexa Pereira (Villanova University); Megan L Povelones (Villanova University)

Trypanosoma brucei undergoes morphological changes to adapt to host environments, including remodeling of their mitochondrial network. In yeast and mammals, membrane remodeling events involve both classical dynamins and dynamin-related proteins (DRPs), with DRPs mediating organelle division. In contrast, *T. brucei* have only one multifunctional dynamin-like protein (TbDLP) with roles in endocytosis and mitochondrial fission. Currently there are no known interacting proteins for TbDLP, and uncovering its molecular mechanism remains complicated by its involvement in multiple cellular pathways. Using tandem affinity purification in the related kinetoplastid Crithidia fasciculata, we have identified 30 putative DLP interacting proteins, almost a third of which have been found associated with glycosomes, glycolytic organelles related to peroxisomes. To explore the role of TbDLP in glycosome structure, we localized a glycosomal marker in procyclic form *T. brucei* during TbDLP RNAi and overexpression. While control and TbDLP knockdown cells showed a granular pattern, overexpression of TbDLP caused an increase in cytoplasmic signal, indicating a defect in glycosomal import. Intriguingly, overexpression of TbDLP also alters its cellular localization, changing from cytoplasmic puncta to accumulations in the periflagellar pocket region. When we probed *T. brucei* lysates for endogenous TbDLP by western blot, we found two bands of similar size, possibly representing modified forms. While both bands decrease during RNAi, overexpression of TbDLP increases only the lower band. We favor a hypothesis whereby overexpression of TbDLP alters its post-translational modification and therefore its subcellular localization, providing a possible mechanism for the spatial regulation of TbDLP function.

Session II Abstracts – Host-Pathogen/Drug Discovery

Keith Gull, Chair
Wednesday 8:45 AM-11:30 AM
September 14th 2022

2A How trypanosomes tell fat to get lost

Henrique Machado (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Peter Hofer (Institute of Molecular Biosciences, University of Graz); Rudolf Zechner (Institute of Molecular Biosciences, University of Graz. BioTechMed-Graz); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

Trypanosoma brucei colonizes the interstitial spaces of the adipose tissue in disproportionately high numbers relative to other tissues. Loss of adipose tissue mass, and more broadly of total body weight, is a hallmark of African trypanosomiasis. Nevertheless, it is unclear if this loss of fat mass is due to colonization of the adipose tissue by *T. brucei* and if fat loss contributes to pathology. Here we show that a *T. brucei* infection results in an increased triglyceride hydrolysis in the adipocytes (i.e. adipocyte lipolysis) through adipose triglyceride lipase (ATGL) activity. We found that adipocyte lipolysis is induced primarily by immune signals. Moreover, adipocyte-specific ATGL deficient mice are resistant to *T. brucei* induced loss of fat mass but succumb earlier to infection, indicating that fat loss is due to ATGL-mediated lipolysis and this process has a protective effect on the host. Interestingly, loss of ATGL activity prevents parasite clearance from the adipose tissue following the first peak of parasite burden. Consistently, chemical activation of adipocyte lipolysis of in vitro grown adipocytes prevents the growth of co-cultured *T. brucei*. Collectively, our results provide mechanistic insight into the loss of fat mass in African trypanosomiasis and identifies adipocyte lipolysis as a host-protective mechanism during a *T. brucei* infection.

2B Developmental incompetence in selected and naturally occurring trypanosome isolates

Guy Oldrieve (The University of Edinburgh); Mathieu Cayla (The University of Edinburgh); Frederik Van den Broeck (KU Leuven); Keith R Matthews (The University of Edinburgh)

Trypanosoma brucei has two distinct life stages in its mammalian host. The proliferative 'slender' form develops into a cell-cycle arrested 'stumpy' form at high parasite density. This transition occurs in a density-dependent quorum sensing (QS) like process, for which key molecular regulators have been identified. Naturally occurring *T. brucei* subspecies (*T. b. evansi* and *T. b. equiperdum*) are unable to generate the stumpy form and are described as 'monomorphic'. Utilising whole genome sequences of 41 naturally occurring monomorphic isolates, we corroborate previous studies in identifying at least four independent monomorphic *T. brucei* clades. Sequence variants unique to these monomorphic lineages were then synthesised and used to replace wild type alleles, via CRISPR-Cas9, in developmentally competent *T. brucei*. Confirming a role for the variant in monomorphism, replacement of one of the mutant targets analysed to date, APPBP1, reduced the ability to generate stumpy forms in developmentally competent cells. Further variants are being tested using this analytical pipeline. We also selected new monomorphic cell lines by laboratory passage from a pleomorphic population and detected significant downregulation of ZC3H20 and GPR89 transcripts associated with the progression to monomorphism. In vitro overexpression of ZC3H20 in the selected monomorphic cells restored pleomorphism. Our results suggest monomorphism can initially arise via transcriptional changes, which can be reversed. Mutations then accrue in key quorum sensing genes which lock the parasites in a monomorphic phenotype. This provides insight into the molecular control of trypanosome QS and diagnostic tools to anticipate increased virulence in the field.

2C Unexpected plasticity in the life cycle of *Trypanosoma brucei*

Jaime N Lisack (Universität Würzburg); Carina Praisler (Universität Würzburg); Sarah Schuster (Universität Würzburg); Markus Engstler (Universität Würzburg)

The digenetic life cycle of African trypanosomes alternates between a mammalian host and an insect vector, the tsetse fly. In the mammalian host, *T. brucei* can be found in two main stages, the proliferative long slender form and the cell cycle arrested short stumpy form. The change from slender to stumpy trypanosomes occurs via a quorum sensing mechanism, dictated by the parasite-excreted stumpy induction factor (SIF). This development entails morphological and metabolic changes, as well as the expression of the stumpy marker protein associated with differentiation1 (PAD1). It was long thought that slender forms die upon entering the fly and only the pre-adapted stumpy forms would continue the life cycle. We have shown that slender forms do not die upon ingestion, can turn on the PAD1 pathway, and seamlessly transition to the first fly stage, without cell cycle arrest. Co-infection experiments with both fluorescently tagged slender and stumpy trypanosomes will be presented that answer the question if one can outcompete the other. In addition, extensive RNA-sequencing of both slender and stumpy trypanosomes during their transition to the first fly form have been conducted. The results not only hold implications regarding the life cycle of *T. brucei* but also on transmission dynamics. This data could help answer the long-standing question of how disease incidence is sustained, despite the absence of stumpy trypanosomes at low blood parasitemia, which is characteristic of chronic mammalian infections.

2D When is a slender not a slender? Assessing terminal commitment to arrest in acute and chronic *Trypanosoma brucei* infections

Stephen D Larcombe (University of Edinburgh); Emma M Briggs (University of Edinburgh); Balazs Szoor (University of Edinburgh); Keith R Matthews (University of Edinburgh)

The development of *T. brucei* in mammals is marked by morphological and physiological change as parasites differentiate from proliferative “slender” to arrested “stumpy” forms. Despite recent progress in understanding the mechanisms driving stumpy formation, basic questions remain unanswered. For example, definitive experimental evidence for the irreversibility of stumpy formation and arrest is limited and any differences between the developmental forms in early and chronic infections unknown. Also, while chronic infections are dominated by intermediate/stumpy forms, how the replicative slender population contributes to the chronic infection profile is unclear. We used RNAi against HYP2 - an effector of stumpy formation - in acute and chronic infections to enrich for slender parasites when these are naturally scarce in vivo. We tracked morphological, physiological and transcriptomic markers of differentiation, and used an ex vivo replating assay as a proxy for commitment to arrest. For acute phase parasites, PAD1 expression was a robust predictor of commitment to arrest, and PAD1 negative slender cells proliferated efficiently ex vivo. In chronic infections, however, only a small proportion of morphologically slender cells could replicate ex vivo, with HYP2 knockdown demonstrating this is not due to host adaption. Hence, most bloodstream slender cells in chronic infections are already committed to arrest, with the replicative slender population being a small subset of these, despite their importance for maintaining the parasite population and generation of new antigenic variants. We are currently investigating molecular mechanisms behind the differences between acute and chronic form parasites using scRNAseq.

TT2E A quantitative mass spectrometry approach to identify novel proteins involved in macrophage infections by *Leishmania* parasites

Nicolas Hagedorn (Department of Cell and Developmental Biology, Würzburg, Germany); Albert Fradera Sola (Institue of Molecular Biology, Mainz); Elisa Theres Rauh (Department of Cell and Developmental Biology, Würzburg, Germany); Tobias Gold (Microbiology Institute-Clinical Microbiology, Erlangen); Ulrike Schleicher (Microbiology Institute-Clinical Microbiology, Erlangen); Falk Butter (Institue of Molecular Biology, Mainz); Christian J. Janzen (Department of Cell and Developmental Biology, Würzburg, Germany)

Leishmania are single-celled parasites with an intracellular life cycle stage in phagocytic cells of their vertebrate hosts. Several transcriptomic analyses of host cell defense mechanisms and how the parasites respond to these processes are already available. Due to the prevalence of post-transcriptional regulation in kinetoplastids however, transcriptomes do not reflect the protein composition very well. Here, we present a systematic comparative in vitro study of the infection process using a quantitative proteomics approach. Murine bone marrow-derived macrophages (BMDMs) were infected with 3 different *Leishmania* species *(L. mexicana*, *L. major*, *L. infantum*). Whole cell protein extracts of parasites and BMDMs were prepared at 7 different timepoints post-infection (0.5-72 h) and were analysed using quantitative mass spectrometry. We were able to quantify the expression of approximately 2000 murine proteins in all 3 infection experiments, while 1500, 1000 and 1400 proteins were analysed after infection with *L. mexicana*, *L. major* and *L. infantum*, respectively. The number of differentially-expressed proteins during the time course of infection varied for each species (349, 133, and 287 proteins for *L. mexicana*, *L. major* and *L. infantum*, respectively). Our experiments identified many proteins putatively involved in the infection process, which were not found in previously published transcriptome analyses. For example, gene ontology enrichment showed 41 *L. mexicana* protein IDs involved in oxidoreductase activity. Additionally, we found 24 differentially-expressed proteins without any functional or structural annotation in the TriTryp database. We are currently investigating several candidate proteins to discover novel mechanisms involved in parasite adaptation processes during macrophage infections.

TT2F Trypanosome sequestration and immune response drive acute cerebral disease

Sara Silva Pereira (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Mariana De Niz (Institut Pasteur); Karine Serre (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Joana Coelho (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Marie Ouarné (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa "); Cláudio A Franco (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

Trypanosoma congolense causes different pathologies in animals, in Africa. Cerebral trypanosomiasis is an acute form of disease, but the mechanism underlying its severity remains unknown. Using a multidisciplinary approach, we developed a mouse model of acute cerebral trypanosomiasis and characterized the cellular, behavioral and physiological consequences of this infection. By intravital microscopy, we showed that *T. congolense* 1/148 strain parasites heavily sequester in the brain vasculature for long periods of time (up to 8 hours). Endothelial-specific ribosome profiling showed that parasite presence results in a strong pro-inflammatory response. We further observed that mice infected with this strain displayed significant and quantifiable neurological clinical signs, which were associated with an overwhelming ICAM1-mediated, CD4+ T cell extravasation into the brain parenchyma. Antibody-mediated ICAM1 blocking and lymphocyte absence reduced parasite sequestration in the brain and prevented the onset of cerebral trypanosomiasis. Furthermore, adoptive cell transfer experiments showed that CD4+ T cells, alone, are sufficient to cause the neuropathology that culminated in mouse death. We propose a mechanism whereby acute cerebral trypanosomiasis depends both on parasite (sequestration) and host factors (ICAM1 and CD4+ T cells). This is a new experimental model that adds novel tools to understanding trypanosomiasis. It will allow researchers to understand the variety of different clinical outcomes in *T. congolense* infections.

TT2G Development of a molecular diagnostic toolbox for the detection of trypanosomatid parasites by SHERLOCK

Elena Pérez-Antón (Institut Pasteur, Université Paris Cité); Roger Junior Eloiflin (Institut de Recherche pour le Développement (IRD)-CIRAD); Annick Dujeancourt-Henry (Institut Pasteur, Université Paris Cité); Aïssata Camara (Institut Pasteur of Guinea); Jean-Mathieu Bart (Institut de Recherche pour le Développement (IRD)-CIRAD); Brice Rotureau (Institut Pasteur, Université Paris Sorbonne; and Institut Pasteur of Guinea); Lucy Glover (Institut Pasteur, Université Paris Cité)

The availability of highly sensitive and specific diagnostic tools, adapted to point-of-care, is crucial for the successful control of infections caused by trypanosomatid parasites. We have adapted SHERLOCK (for Specific High-sensitivity Enzymatic Reporter unLOCKing (Kellner et al. 2019; PMID: 31548639) for the rapid and specific detection of trypanosomatid parasites. SHERLOCK combines pre-amplification of nucleic acids followed by specific recognition of the desired RNA regions by the CRISPR-Cas13a system. Targeting the *Trypanosoma brucei gambiense*-specific glycoprotein (TgSGP) and the T. b. rhodesiense-specific serum resistance associated gene (SRA) allowed us to discriminate between the two species with a higher sensitivity compared to qPCR. We have now optimized SHERLOCK to detect most human-infective trypanosomatid species using an 18S guide by selecting conserved sequences among different trypanosomatid species - Pan-Trypanozoon or Pan-trypanomatid (including at least Trypanozoon subgenus, *Leishmania* sspp. and *T. cruzi*), or non-conserved 18S sequences for the detection of *T. congolenese* or *T. vivax*. Furthermore, to achieve a point-of-care test, we are optimizing the SHERLOCK technology by adapting the pre-amplification step from RPA to LAMP, as well as modifying the reaction conditions to achieve a more cost-effective technique with less equipment and pipetting, and less time consumed.

TT2H Fexinidazole survival genes implicate novel redox pathway in drug resistance

Arthur Drouaud (George Washington University); Jenna Murrow (George Washington University); Galadriel Hovel-Miner (George Washington University)

Fexinidazole has emerged as the front-line treatment against African trypanosomiasis, offering the first oral monotherapy against *T. brucei gambiense* infections. Because of its current success in African trypanosome treatment, fexinidazole is actively being investigated as a therapy against American trypanosomiasis (Chagas) and Leishmaniasis, which currently infect over 15 million people globally. Fexinidazole joined nifurtimox (NECT combination therapy, HAT) and benznidazole (Chagas disease) as clinically significant nitroaromatic compounds for the treatment of trypanosomatid infections. Nitroheterocyclic compounds function as prodrugs that require nitroreductase (NTR) enzymatic activation to generate cytotoxic species that are thought to damage DNA, lipids, and proteins. Naturally occurring and laboratory resistance and cross-resistance have been documented based on *T. brucei* NTR mutants and in RNAi-based knock-down studies, respectively. Fexinidazole is a repurposed compound whose success in clinical trials enabled rapid approvals with few publications evaluating the drugs trypanocidal phenotypes, Mechanism of Action (MoA), or potential for drug resistance. Inadequate molecular understanding of fexinidazole's MoA and modes of drug resistance represent a significant knowledge gap. To fill this gap, our state-of-the-art *T. brucei* Gain-of-Function library was applied to the question of fexinidazole resistance and preliminary studies identified 11 survival associated ORFs including oxidoreductases, a peroxidase, dithiol glutaredoxin, and a glutathione S-transferase. Collectively, the identified genes support the hypothesis that novel fexinidazole survival genes constitute a redox-based pathway able to promote nitroaromatic drug resistance with implications for fexinidazole's MoA.

TT2I A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease

Tamara Sternlieb (Institute of Parasitology, McGill University); Rhiannon Heslop (Institute of Parasitology, McGill University); Mira Loock (Institute of Parasitology, McGill University); Luisa Berenguer Antunes (Institute of Parasitology, McGill University); Igor Cestari (Institute of Parasitology, McGill University;Division of Experimental Medicine, McGill University)

Chagas disease (ChD) affects about 8 million people and is caused by infection with the protozoan parasite Trypanosoma cruzi. The infection results in an acute disease with flu-like symptoms that often develop into a chronic and lethal cardiac disease. There are no vaccines for ChD. The available drugs, benznidazole or nifurtimox, are highly toxic and their efficacy is limited. To identify potential vaccine targets for ChD, we generated a *T. cruzi* genome-wide library for yeast surface display (YSD) for screening parasite proteins that react with ChD patient's antibodies. The genome-wide library fragments range from 0.5 to 3 kb and represents 24-fold of the parasite genome. Nanopore sequencing confirmed that the library covered all chromosomes, and each parasite gene was represented by multiple clones. Computational prediction of the library-expressed proteins showed over 83,000 unique polypeptides, of which some might help identify immunogenic epitopes. Using western blot, flow cytometry, and imaging analysis we confirmed the surface expression of library proteins in yeast. Analysis of the *T. cruzi* YSD library against chronic ChD patients' sera showed that 5-10% of the yeast reacted with patients' antibodies, whereas about 1% reacted with sera of healthy subjects. Three rounds of YSD enrichment with patients' sera using magnetic-assisted cell sorting resulted in ~7-fold enrichment of the library with ~40% of the YSD population reacting with ChD patient antibodies. Using nanopore sequencing, we are identifying the *T. cruzi* antigens targeted by chronic ChD patient antibodies. We will validate the identified antigens as potential vaccine targets in animal models.

TT2J Structure-guided discovery of a small molecule inhibitor targeting *Trypanosoma brucei* Replication Protein A1

Aditi Mukherjee (Rutgers University, PHRI); Zakir Hossain (Queens College); Junyong Choi (CUNY Graduate Center, Queens College); Hee-Sook Kim (Rutgers University, PHRI)

DNA replication proteins are attractive targets for cancer therapies and antimicrobials because of their essential functions in cellular proliferation and viability. Replication protein A (RPA), consisting of RPA1, 2 and 3 subunits, is a eukaryotic ssDNA-binding protein and is essential for DNA replication and repair. *T. brucei* also contains all three subunits of RPA complex. Given that TbRPA1 has only 36% sequence similarity to human RPA1, we reasoned the feasibility of identification of TbRPA1-specific inhibitors. From the structure modeling and induced-fit docking analysis, we discovered a small molecule inhibitor of TbRPA1 (JC-229). The inhibitor targets the ssDNA-binding activity of TbRPA1, exhibiting 25 nM and 240 nM IC50 on purified TbRPA complex and TbRPA1 DNA-Binding-Domain (DBD) A&B (TbRPA1-DBD-AB), respectively. Interestingly, JC-229 showed no inhibition to the purified HsRPA complex or HsRPA1-DBD-AB even at 100 μM. Surprisingly, JC-229 showed no inhibitory effect on purified *T. cruzi* or *L. mexicana* DBD-AB proteins, although they have ~80% sequence identity. From the molecular modeling of JC-229 with Tb, Tc, and LmexRPA1-DBD-AB, we found that TbRPA1-Ser105 residue is responsible for this discrepancy and biochemically confirmed that the domain A of TbRPA1 contains a JC-229-binding pocket. JC-229-treated *T. brucei* cells behaved similarly as TbRPA1-depleted cells, exhibiting a strong inhibition of DNA synthesis. Lastly, JC-229 exhibited 6.6 μM EC50 on *T. brucei* growth, while showing ~ 20 μM and ~ 40 μM EC50 on HEK293 and HeLa cells, respectively. Here, we will discuss about JC-229's potential as a therapeutic tool for the trypanosomiasis treatment in the future.

Session III Abstracts – Gene Expression I

Monica Mugnier, Chair
Wednesday 2 PM-4:15 PM
September 14th 2022

3A Modified length of the tRNA Trp anticodon stem enabled the UGA stop codon reassignment across species.

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Genetic code was previously thought to be universal for all the life forms but soon variations from canonical code were described in many organisms all across the tree of life, most of the deviation involves reassignment of one or two stop codons to sense codons leaving 1 or 2 stop codons to confer the termination of translation. Interestingly, in 2016 two groups of protist trypanosomatid Blastocrithidia nonstop (B.nonstop) and several ciliates were shown to reassign all the three stop codons in their nuclear genome to code amino acids. Transcriptome and proteome analysis of B.nonstop revealed UGA has been reassigned to code tryptophan, while UAG and UAA are reassigned to code glutamate. Robust comparisons with other trypanosomatids revealed specific features at the end of the coding regions defining UAA as the only functional termination codon. We demonstrate that while novel tRNAGlu fully cognate to UAG and UAA evolved to reassign these two stops, recoding of UGA followed an unprecedented path via shortening the anticodon stem-loop of tRNATrp CCA from 5 to 4 base pairs (bp). While tRNATrp with a canonical 5-bp long stem-loop recognizes UGG as dictated by the genetic code, its shortened version efficiently incorporates tryptophan also into in-frame UGA to allow translation to continue. We have shown that this alteration has a functionally universal character as it is experimentally transferrable to S. cerevisiae and the human parasite *T. brucei*. Importantly, we also demonstrate that the evolutionary unrelated ciliate C. magnum has independently adopted the same strategy. Our research opens a new route for synthetic biology in genetic code manipulations and challenges our current understanding of very fundamental process of life “Translation”.
Publication: Kachale, A. et al., (2022) Modified length of the tRNA Trp anticodon stem enabled the UGA stop codon reassignment across species. Nature (under revision).

3B Identification of novel anti-sense regulators in *Trypanosoma brucei*

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In the absence of microRNAs in trypanosomes it was unknown if anti-sense regulators exist in these parasites. Recently, our groups described two such regulators, TBsRNA-33 and TBsRNA-37, that associate with ribosomes and polyribosomes and interact with distinct mRNA to regulate their translation (Rajan et al. iScience 2021). In addition, ~1,500 long non-coding RNAs (lncRNA) were described in *T. brucei*. One of these is processed to a C/D small nucleolar RNA termed snoGRUMPY. snoGRUMPY acts as an anti-sense regulator of mRNAs involved in differentiation from slender to stumpy bloodstream forms (Guegan et al., Sci. Adv. 2022). We will present data on additional C/D snoRNAs that, like snoGRUMPY, act as anti-sense regulators of mRNAs. TBsRNA-23 was identified in our screen for ncRNAs; it is located between GPEET and EP3 procyclin genes. TBsRNA-23 undergoes trans-splicing and polyadenylation; its silencing by RNAi decreased the level of GPEET mRNA and protein and increased the level of EP mRNA and protein, while overexpression of the RNA had an opposite effect. The base-pair interaction between TbsRNA-23 and the 3' end of GPEET mRNA was verified by demonstrating that overexpression of TBsRNA-23, mutated in the interaction domain, lost its ability to affect GPEET and EP levels. TBsRNA-23 was shown to cross-link in vivo to transcripts that are differentially expressed in early and late procyclic forms. These differ in social motility. Cells overexpressing TBsRNA-23 are hypermotile. Purification of factors associated with TBsRNA-23 also identified proteins that are differentially expressed in early versus late procyclic forms. We propose the TbsRNA-23 regulates the transition between these two stages by modulating translation, thus controlling social motility.

3C Role of the C- and N-terminal extensions of the *T. brucei* mRNA decapping enzyme ALPH1 in enzyme localisation, dimerization and protein interaction

Susanne Kramer (University of Würzburg); Natalia Karolak (University of Warsaw); Paula A.C. Londoño (University of Würzburg); Anna Krylova (Charles University in Prague); Johanna Odenwald (University of Würzburg); Claudia Moreira (University of Würzburg); Amelie Eden (University of Würzburg); Carina Goos (University of Würzburg); Bernardo Gabiatti (University of Würzburg); Andrea Reichert (University of Würzburg); Mark Carrington (University of Cambridge); Maria Górna (University of Warsaw); Martin Zoltner (Charles University in Prague)

Kinetoplastids encode no homologues of canonical decapping enzymes. Instead, we identified the ApaH-like phosphatase ALPH1 acting as the major mRNA decapping enzyme in *Trypanosoma brucei*. Absence of this protein family from mammalian systems renders ALPH1 an attractive drug target, which encouraged us to devise a biochemical assay to monitor ALPH activity and to initiate a screening campaign to identify inhibitors with potential as highly selective next generation drugs. Further, we investigated the functions of the N- and C-terminal extensions adjacent to the *T. brucei* ALPH1 catalytic domain, which are devoid of conserved motifs or domains. The predicted unstructured N-terminal region mediates ALPH1 localisation to a granule at the posterior pole of the cell but is not required for localisation to starvation stress granules. The C-terminus, containing a predicted, all helical domain, mediates ALPH1 dimerization in vitro and is required for efficient localisation to both the PP granule and starvation stress granules. Proximity labelling identified a cohort of several high-confidence interactors, including two proteins, whose interaction with ALPH1 is dependent on the presence of the C-terminal region: the 5´-3´ exoribonuclease XRNA and a protein kinase. Significantly, both enzymes share ALPH1 distinct granule localisation and could be confirmed by reverse interactomics approaches. Altogether, our data demonstrates that the C-terminus plays a major role in ALPH1 cellular function and regulation, consistent with its conservation across the Kinetoplastida, and strongly suggests that ALPH1 is acting in a decapping complex.

3D Using metabolic labeling and scRNA-seq to understand gene expression in *Trypanosoma brucei*

Vanessa Luzak (LMU Munich); Raúl O. Cosentino (LMU Munich); Atai Dobrynin (LMU Munich); Maria Colomé-Tatché (LMU Munich); Florian Erhard (JMU Würzburg); T. Nicolai Siegel (LMU Munich)

Gene expression is a multi-step process and often analyzed by RNA-seq, a method measuring steady state mRNA levels from millions of cells. Hereby, important information is lost, such as the contribution of individual gene expression steps (i.e. transcription, mRNA processing and degradation) to steady state RNA levels, as well as information from single cells. Trypanosomes have evolved particular gene expression regulation, which seems to differ strongly from other eukaryotes. However, how exactly trypanosome gene expression is regulated remains poorly understood. To address this question, we have established metabolic labeling of nascent RNA in bloodstream form parasites by genetic engineering. We can now perform Transient Transcriptome sequencing (TT-seq) to measure transcription and mRNA processing rates by ultra-short labeling, as well as SLAM-seq to determine mRNA half-lives without inhibiting essential cellular processes. Using these two approaches, we aim to identify regulatory steps in trypanosome gene expression. In single cells, gene expression is a highly stochastic process and numerous studies have shown that only a subset of genes is expressed per cell at any time – a process referred to as transcriptional bursting. By mapping scRNA-seq data to an allele-specific genome assembly, we are now able to study transcriptional bursting in trypanosomes. We aim to understand whether bursting occurs in the context of ubiquitous transcription initiation and genes organized in PTUs. Further, we analyze if bursting of the active VSG gene is prevented to guarantee each cell expressing sufficient VSG levels at any time? In summary, we combine two high-resolution approaches, namely metabolic labeling and scRNA-seq, to better understand how gene expression is regulated in trypanosomes. In future, metabolic labeling directly upstream of scRNA-seq analysis will enable even higher resolution data for individual cells.

3E deGradFP for an efficient way to degrade target proteins in *Trypanosoma brucei*

Midori Ishii (University of Oxford); Bungo Akiyoshi (University of Oxford)

Conditional knockdown of proteins of interest is an invaluable tool to analyze their functions in cells. Conditional knockout by Cre-LoxP or CRISPR and conditional knockdown by RNA interference are widely used in *Trypanosoma brucei*. These methods are powerful, but they have limitation that protein stability affects the efficiency of depletion. To overcome this problem, we adapted a targeted protein degradation system, deGradFP (degrade Green Fluorescent Protein) to *T. brucei*. This method uses an anti-GFP nanobody to target GFP-fusion proteins to an endogenous ubiquitin-proteasome pathway. We expressed the N-terminal part of a putative F-box protein FBP75 (Tb927.5.700) with anti-GFP nanobody VhhGFP4 using the doxycycline inducible system. We tagged both alleles of our target proteins with yellow fluorescent protein (YFP) in one transfection step. To test deGradFP, we first targeted a nuclear protein, kinetoplastid kinetochore protein KKT3. KKT3 is estimated to be a very stable protein, which makes it difficult to get efficient depletion by the RNAi system. We added nuclear localization signal to deGradFP (NLS-deGradFP) to efficiently target it inside the nucleus. Induction of NLS-deGradFP on KKT3-YFP cells caused more severe growth defect than RNAi. Microscopy analysis confirmed that KKT3-YFP signal was clearly decreased at 6 hours after NLS-deGradFP induction. We next tested deGradFP without NLS for a cytoplasmic protein, COPII subunit, SEC31-YFP. The growth defect caused by deGradFP was much stronger than previously reported RNAi. These results show that deGradFP works for both nucleus and cytoplasmic proteins in *T. brucei*. NLS-deGradFP and deGradFP plasmids are available upon talking to me (first 10 people).

TT3F Investigating trans-regulators of the *Leishmania* lifecycle

Ewan Parry (University of York); Natalia M.M. Teles (University of York); Rachel Neish (University of York); Katherine Newling (University of York); Michael J. Plevin (University of York); Angela K. Cruz (University of São Paulo, Ribeirao Preto); Jeremy C. Mottram (University of York); Pegine B. Walrad (University of York)

Leishmaniasis cases are rising globally, no vaccine is currently available and limited treatments have toxic side effects and growing resistances. *Leishmania* parasites transition between extracellular stages in the sandfly to an intracellular stage that infects and destroys human immune cells. Fundamental parasite processes can inform new intervention strategies. Lifecycle progression requires tightly-coordinated gene expression that preadapts *Leishmania* parasites for survival and virulence. Kinetoplastid gene regulation is predominantly reliant on post-transcriptional mechanisms. Accordingly, RNA binding proteins (RBPs) have an elevated importance for gene regulation in these organisms. Despite this, strikingly few trans-regulators have been characterised in *Leishmania* to date. Recently we isolated and compared the mRBPome of the 3 main *Leishmania* lifecycle stages and found that RBPs selectively bind distinct transcriptomes in a stage-specific manner. Moreover, a separate study revealed that arginine methylation of RBPs impacts target transcript association and stability in a method both bespoke and precise with epigenetic implications toward parasite virulence. Building upon this work, a bar-coded trans-regulator knockout clone library was created and screened for essential roles in lifecycle progression and macrophage/mouse infections. Of those screened, 40 RBPs were incapable of creating viable knockout cell lines and 18 contribute to cell differentiation to human-infective stages and/or parasite infectivity. Examination of individual knockout lines for amastigote-specific RBPs showed normal promastigote growth dynamics, whereas infection in peritoneal macrophages was inhibited. This suggests essential roles for these RBPs in amastigote viability and virulence. Experiments are underway to identify the novel virulence pathways these RBPs regulate. Our findings link *Leishmania* trans-regulatory networks to eukaryotic post-transcriptional epigenetics.

TT3G Genome-scale RNA interference profiling of *Trypanosoma brucei* cell cycle progression defects

Melanie Ridgway (University of Glasgow); Catarina A Marques (University of Glasgow); Michele Tinti (University of Dundee); Andrew Cassidy (University of Dundee); David Horn (University of Dundee)

Trypanosomatids, which include major pathogens of humans and livestock, are divergent eukaryotes for which cell cycle controls and the underlying mechanisms are not completely understood. Here, we describe a genome-wide RNA-interference library screen for cell cycle defects in *Trypanosoma brucei*. We induced massive parallel knockdown, sorted the perturbed population using high-throughput flow cytometry, deep-sequenced RNAi-targets from each stage and digitally reconstructed cell cycle profiles at a genomic scale; also enabling data visualisation using an online tool (https://tryp-cycle.pages.dev/). Analysis of several hundred genes that impact cell cycle progression revealed over 100 flagellar component knockdowns linked to genome endoreduplication, evidence for metabolic control of the G1-S transition, surface antigen regulatory mRNA-binding proteins required for cytokinesis, and a putative nucleoredoxin required for both mitochondrial genome segregation and for mitosis. The outputs provide comprehensive functional genomic evidence for the known and novel machineries, pathways and regulators that coordinate trypanosome cell cycle progression.

TT3H Thousands of messenger RNA untranslated regions reprogram post-transcriptional gene expression profiles in trypanosomes

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

Cells express thousands of proteins that differ in abundance over a wide range. In nucleated cells, messenger RNA untranslated regions (UTRs) can contribute to post-transcriptional expression control, but relatively few UTRs have been shown to exert these controls, and cis-regulatory sequences have remained largely unknown. In the trypanosomatids, several of which are important human and animal parasites, genome-wide polycistronic transcription places a particular emphasis on post-transcriptional controls. Here, we describe a massive parallel reporter assay coupled with UTR-seq profiling in the African trypanosome, revealing post-transcriptional reprogramming by thousands of 3'-UTRs. Genome-scale UTR-seq screening identified an abundance of regulatory fragments that either increased or reduced reporter expression. Analysis of regulatory fragments and native UTRs yielded a correlation between gene expression, 3'-UTR length and adenine (A)-rich poly-purine tract abundance, as well as a more widespread enrichment of thymine (T)-rich poly-pyrimidine tracts in UTRs. A machine learning approach guided by these findings effectively predicted observed measures of translation efficiency at a transcriptomic scale; R2 was 0.69. This approach also provided quantitative measures of the relative contribution of sequences within native 3'-UTR and revealed similarly predictive sequences within 5'-UTRs. Thus, we describe the cis-regulatory UTR sequences that control gene expression in the context of a genome that lacks promoter-based transcription control. We conclude that gene expression profiles in trypanosomes are post-transcriptionally reprogramed by thousands of poly-purine tract biased UTRs.

TT3I The role of KPAP1 methylation in transcript-specific mitochondrial mRNA tail addition in *Trypanosoma brucei*

Clara Smoniewski (University of Minnesota Medical School, Duluth campus); Poorya Mirzavand Borujeni (McGill University); Marshall Hampton (University of Minnesota Duluth); Reza Salavati (McGill University); Sara L Zimmer (University of Minnesota Medical School, Duluth campus)

Trypanosoma brucei mitochondrial mRNAs have non-templated heterogenous adenine/uridine tails added post-transcriptionally to their 3' ends. We previously found population level differences in tail characteristics, such as length and composition, between transcripts. The mechanism(s) for these differences is not established. Tail adenines are added by kinetoplast poly(A) polymerase I (KPAP1), possessing two previously identified arginine methylation sites. Methylation of protein arginine residues is a known mechanism for altering protein:protein and protein:nucleic acid associations. We have mutated KPAP1 at its arginine methylation sites to mimic constant methylation (methyl-mimic) or no methylation (methyl-mutant). These mutated versions of KPAP1 and a wildtype version were inducibly expressed in procyclic *T. brucei* along with simultaneous silencing of native KPAP1. The three distinct cell lines were then compared. Using circTAIL-seq, which combines enzymatic circularization of RNA, RT-PCR and Illumina sequencing, we found that some transcripts' tail characteristics are affected by methyl-mimic or methyl-mutant KPAP1. This suggests a role for methylation in KPAP1 regulation. Surprisingly, some transcripts yielded slightly shorter circTAIL-seq libraries when the ligase responsible for RNA circularization was omitted as a negative control. Sequencing reveals that these libraries consist of cDNAs possessing 3' and 5' UTRs of the transcript of interest, often truncated, sometimes possessing a short string of adenines and/or uridines between the two UTRs. We are currently pursuing additional approaches to determine whether these products reflect a minor sub-population of the mitochondrial mRNAs that are intracellularly circularized. To our knowledge, this would be the first circular RNA to be identified in a Kinetoplastid species.

TT3J Biogenesis of trypanosomal mitoribosomes employs conserved and lineage-specific mechanisms and multitude of assembly factors

Ondřej Gahura (Biology Centre of the Czech Academy of Sciences); Jonathan Wong (Biology Centre of the Czech Academy of Sciences); Prashant Chauhan (Biology Centre of the Czech Academy of Sciences); Shintaro Aibara (Science for Life Laboratory, Stockholm University); Vivek Singh (Science for Life Laboratory, Stockholm University); Alena Zíková (Biology Centre of the Czech Academy of Sciences); Alexey Amunts (Science for Life Laboratory, Stockholm University)

Mitoribosomes in trypanosomatid parasites diverged markedly from their bacterial ancestors and other eukaryotic lineages in terms of protein composition, rRNA content and overall architecture. We and others obtained cryoEM structures of several native precursors of large and small mitoribosomal subunits (mtLSU and mtSSU), which revealed that biogenesis of trypanosomatid mitoribosomes employ intricate machineries containing a multitude of assembly factors. We showed that the assembly of *T. brucei* mtSSU requires a homolog of RbfA, acting in biogenesis of bacterial ribosomes and mammalian mitoribosomes. Conserved core of TbRbfA binds several key rRNA helices of the decoding center and mRNA channel and together with long lineage-specific extensions at both termini stabilizes the immature interface of all four domains of mtSSU rRNA. The C-terminal extension also serves as a binding platform for a heterodimer of two conserved methyltransferases and a kinetoplastid-specific pentameric assembly factor mt-SAF24. The N-terminal domain of mt-SAF24 forms a protrusion on the prospective intersubunit interface, which is capped by a disc-shaped density of unknown composition. By combining in vivo and in vitro approaches, we test the hypothesis that the density represents a piece of phospholipid bilayer and that the biogenesis of mtSSU occurs in the association with the inner mitochondrial membrane. Comparison of the trypanosomal pre-mtSSU complexes with a recently reported series of mammalian mtSSU precursors revealed common assembly mechanisms and allowed us to infer a chimeric pathway for mtSSU assembly and propose that the exit part of the mRNA channel in trypanosomal mitoribosome is deviated compared to other ribosomes.

Session IV Abstracts – Metabolism

Igor Cestari, Chair
Thursday 8:45 AM-11:30 AM
September 15th 2022

4A ELO1 is critical for maintenance of broad metabolic processes and efficient growth in *Trypanosoma cruzi* epimastigotes

Lucas Pagura (Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health); Peter C Dumoulin (Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health); Cameron C Ellis (Department of Biological Sciences Department, University of Texas at El Paso); Igor Estevao (Department of Biological Sciences Department, University of Texas at El Paso); Maria T Mendes (Department of Biological Sciences Department, University of Texas at El Paso); Igor C Almeida (Department of Biological Sciences Department, University of Texas at El Paso); Barbara A Burleigh (Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health)

The mechanisms by which *Trypanosoma cruzi* maintains the optimal membrane lipid composition are not well understood. The fatty acyl (FA) chains used for lipid synthesis/remodeling can be derived de novo, via a microsomal FA elongase (ELO) system, or scavenged from the extracellular environment. *T.cruzi* encodes five ELOs (ELO1-5) that act sequentially or in a modular fashion to elongate: C4:0 to C10:0 (ELO1), C10:0 to C14:0 (ELO2), and C14:0 to C18:0 (ELO3). ELO4 and ELO5 elongate very long chain FA and polyunsaturated FA. To determine the contribution of endogenous FA synthesis toward lipid homeostasis and growth of *T.cruzi*, null mutants were generated in which the genes encoding ELO1-3 were individually disrupted. Lipidome analysis of ∆elo2 and ∆elo3 epimastigotes revealed shortening of the FA chain length in diverse lipids sub-classes, consistent with the role of ELOs in lipid homeostasis. These changes were well-tolerated in epimastigotes as the growth of these mutants was minimally impaired. In contrast, disruption of ELO1 was associated with significant growth impairment but minimal lipidome perturbation. Unlike the other mutants, ∆elo1 epimastigotes exhibited mitochondrial disturbances (decreased membrane potential and fatty acid oxidation; increased mROS production). Metabolic dysregulation in ∆elo1 epimastigotes was further illustrated by marked changes in metabolites associated with glycolysis, pentose phosphate pathway, TCA cycle, and nicotinamide metabolism. Metabolic and growth phenotypes observed in the ∆elo1 mutant were restored by genetic complementation. Thus, ELO1 is unessential for maintaining the lipid composition membranes but critical for homeostatic control of broad metabolic processes and growth of *T.cruzi* epimastigotes. 1. Lee, S.H., et al. (2006). Fatty acid synthesis by elongases in trypanosomes. Cell 126:691-699, doi:10.1016/j.cell.2006.06.045

4B Prophylactic glycan- and peptide-based vaccines elicit a protective immune response and miRNA modulation in a nonhuman primate model of Chagas disease

Priscila Silva Grijo Farani (University of Texas at El Paso); Susana Portillo (University of Texas at El Paso); Miguel A. Beltran (University of Texas at El Paso); Jacqueline Lopez (University of Texas at El Paso); Maria Tays Mendes (University of Texas at El Paso); Uriel Ortega-Rodriguez (University of Texas at El Paso); Igor Estevao (University of Texas at El Paso); Jane VandeBerg (Texas Biomedical Research Institute); Carlos R. Brito (University of Texas at El Paso); Otacilio C. Moreira (Oswaldo Cruz Institute, Oswaldo Cruz Foundation); Laura Isobel McCall (University of Oklahoma); Rosa A. Maldonado (University of Texas at El Paso); Katja Michael (University of Texas at El Paso); John L. VandeBerg (Texas Biomedical Research Institute); Igor C. Almeida (University of Texas at El Paso)

Trypanosoma cruzi trypomastigote surface is densely covered by glycosylphosphatidylinositol (GPI)-anchored glycoproteins (e.g., tGPI-mucins, mucin-associated surface proteins [MASPs], and trans-sialidases). These molecules can be secreted and strongly activate the host immunity, thus being potential targets for immunotherapy. The trisaccharide Galα1,3Galβ1,4GlcNAcα (Galα3LNα) found in the tGPI-mucins has been described to induce protective, parasite-specific anti-α-Gal antibodies in Chagas disease (CD) patients. Here, we assess the efficacy of the synthetic Galα3LNα- (NGP24h) and MASP-based (MASPpep-KLH) vaccines, alone or combined, in the presence of liposomal monophosphoryl lipid-A (L-MPLA) adjuvant, in Papio hamadryas baboons as an experimental model of acute *T. cruzi* infection. The control group received of L-MPLA alone. Immunized baboons (n=4, each group) exhibited increased specific antibody levels against the two vaccines, alone or combined. A significant decrease in heart inflammation was observed in groups vaccinated with NGP24h, MASPpep-KLH, or NGP24h+MASPpep-KLH. Parasitic load in the heart showed a significant reduction in the MASPpep-KLH group, compared to control animals. A cytokine profile array of the cardiac tissues revealed a general inflammatory unbalance in the L-MPLA-vaccinated group compared with noninfected controls, and the NGP24h vaccine could restore TNF-α overexpression to normal levels, indicating a better prognosis. Cardiomyopathy-related microRNAs were assessed and some exhibited upregulation in the L-MPLA group compared with the noninfected controls. Metabolomic profile analysis showed a decrease in detrimental cardiac markers in groups receiving only one of the two vaccine formulations. Taken together, these results could further our understanding of the molecular mechanisms underlying the protection profile conferred by glycan- and peptide-based CD vaccines.

4C ROS is not just SOS: role of reactive oxygen species in metabolic remodeling during *Trypanosoma brucei* differentiation

Michaela Kunzová (University of South Bohemia, Faculty of Science); Eva Doleželová (Biology Centre CAS, Institute of Parasitology); Alena Zíková (Biology Centre CAS, Institute of Parasitology)

During the cellular differentiation of *Trypanosoma brucei*, the parasite undergoes a dramatic metabolic switch from oxidative phosphorylation in the insect forms to aerobic glycolysis in the bloodstream forms. This remodeling is accompanied by changes in the structure and physiology of the parasite's single mitochondrion. In general, this dynamic organelle is capable of releasing signaling molecules, such as reactive oxygen species (ROS), to regulate various cell processes. To elucidate the role of ROS in the metabolic remodeling of trypanosomes, we use an in vitro differentiating cell line overexpressing RNA binding protein 6 (RBP6). During this in vitro induced differentiation, we observed striking changes to the mitochondrial bioenergetics, which indeed manifested in elevated ROS levels. The changes in the ADP/ATP ratio and higher ROS levels led to the activation of AMP activated protein kinase (AMPK), the main sensor of cellular energy status. Using exogenously expressed mitochondrial and cytosolic catalases, we demonstrated that mitochondrial ROS molecules are essential for AMPK activation and for efficient parasite developmental progression to metacyclic stage. Serendipitously, we have also generated an RBP6 overexpressing cell line that is void of the inhibitory peptide 1 that regulates FoF1-ATP synthase activity. Consistent with our hypothesis on ROS-driven differentiation, these parasites exhibited even higher ROS levels, underwent more efficient differentiation into metacyclic stage and demonstrated earlier AMPK activation. Notably, only these cells were able to differentiate into the bloodstream form in vitro. In summary, our data suggest that ROS are essential signaling molecules that reinforce in vitro differentiation of *T. brucei*.

4D *Trypanosoma brucei* bloodstream form mitochondrion is an ATP producing organelle

Gergana Taleva (Biology Centre CAS); Michaela Husova (Biology Centre CAS); Brian Panicucci (Biology Centre CAS); Carolina Hierro Yap (Biology Centre CAS); Martin Moos (Biology Centre CAS); Petr Simek (Biology Centre CAS); Falk Butter (IMB Mainz); Frederic Bringaud (University of Bordeaux); Alena Zíková (Biology Centre CAS)

Over the last 30 years, the bioenergetic dogma of the *Trypanosoma brucei* bloodstream stage revolved around the central tenet that the mitochondrion is strictly an ATP-consuming organelle and glycolysis is the sole source of cellular ATP. Under this guiding principle, the mitochondrial ADP/ATP carrier (AAC) is presumed to transport cytosolic ATP into the matrix of the organelle, where it is hydrolyzed by FoF1-ATPase to generate the essential mitochondrial membrane potential. However, contrary to expectations, we generated AAC null mutants that remain fully viable in culture and virulent in a mouse model. Importantly, the mitochondrial membrane potential of these parasites is still maintained by FoF1-ATPase, while functional assays do not reveal any other mitochondrial ATP carriers. Intriguingly, the AAC null mutants are more sensitive to an inhibitor of enzymes immediately upstream of an ATP-producing succinyl Co-A synthetase (SCoAS), suggesting that mitochondrial substrate phosphorylation pathways are active. Indeed, SCoAS null mutants are less virulent in vivo, have lower mitochondrial ATP levels and now require AAC activity to import ATP into the mitochondrial matrix. Furthermore, metabolomic analyses indicate perturbations in ATP-generating metabolic pathways leading to acetate and succinate production. Notably, parasites cultivated in low glucose or glycerol containing media exhibited a decreased mitochondrial membrane potential and a significant growth phenotype when SCoAS was rapidly depleted by RNAi. Comprehensively, these new findings revise the original view of a dormant bloodstream form mitochondrion by highlighting the metabolic complexity of the organelle that may enable the parasite to successfully colonize diverse environments within the mammalian host.

4E Tropism, immunity and pathology in the murine model of African trypanosomiasis

Chloe Barnes (Lancaster University); Nadin Fathallah (Lancaster University); John Worthington (Lancaster University); Lucy Jackson-Jones (Lancaster University); Neil Dawson (Lancaster University); Michael Urbaniak (Lancaster University)

Trypanosoma brucei is the cause of human and animal African trypanosomiasis, and survives in the mammalian host by evading the innate and acquired immune system. In stage I of an infection the parasite resides in the blood, lymph and a variety of other tissues whilst in stage II the parasites enter the brain causing severe neurological disturbances. In culture *T. brucei* depletes tryptophan from the media and excretes unused biochemical intermediates, and metabolomics of stage II HAT patients has confirmed a significant decrease in tryptophan in the CSF. As tryptophan is the precursor of both serotonin/melatonin and kynurenine pathways, metabolism of tryptophan by trypanosomes and excretion of metabolites in the brain may interfere with host neurotransmission. We have used a bioluminescent murine model to correlate parasite tropism with changes in host behavior, and have replicated tryptophan depletion and observed perturbed brain metabolism. Novel parasite localisation detected in the gut was coupled with reduced regulatory T-cells and significant enlargement of the omentum and expansion of the omental immune cell population. RNAseq analysis of tissues from naive and infected mice at stage I and II revealed tissue specific differences in the host response to infection. The gut is the major source of tryptophan in mammals, and there is growing recognition of the importance of the direct interaction between the brain and the gut through the gut-brain axis. These results therefore provide a potential link between infection, immune response and perturbed brain function.

4F Probing Fatty Acid Uptake Mechanisms in *Trypanosoma brucei*

Mufida Ammar (Clemson University); Joshua Saliutama (Clemson University); Nava Poudyal (Clemson University); Kristina Parman (Clemson University); Brianna Volbeer (Clemson University); Virginia Dellinger (Clemson University); Bailey Holder (Clemson University); Solangie Pulido-Gomez (Clemson University); Courtney Fant (Clemson University); Dan Spangler (Clemson University); Kimberly Paul (Clemson University)

Trypanosoma brucei readily acquires a range of lipids (from fatty acids to sterols) from its mammalian and tsetse fly hosts. However, little is known about the molecular mechanisms mediating fatty acid uptake in *T. brucei* and its contribution to parasite survival. Given the redundancy of the apparent *T. brucei* uptake repertoire, we undertook a biochemical characterization of fatty acid uptake in the bloodstream (BF) and procyclic form (PF) of *T. brucei* using fluorescent BODIPY-conjugated fatty acids. We found that BODIPY-fatty acid uptake was partially saturable (BODIPY-C16 but not -C12), was impaired below 4°C, and exhibited a preference for longer-chain fatty acids than shorter ones. We assessed the impact of protein-mediated uptake by digesting surface proteins with Proteinase K and found that surface proteolysis did not affect BODIPY-C16 or -C12 uptake. Reduction of cellular ATP levels by 80-90% with metabolic inhibitors (2-deoxyglucose ± sodium azide) reduced BODIPY-C12 uptake by 70%. Finally, we assessed the effect of Triascin C, an inhibitor of Acyl-CoA Synthetases (ACSs), on fatty acid uptake and cell viability. ACS catalyzes the ATP-dependent esterification of fatty acids to Coenzyme A. Triascin C inhibited BODIPY-C12 uptake in BFs and PFs with EC50s of 1.2 μM and 0.8 μM, respectively, with maximal inhibition of 10-30%. Triascin C killed both BFs and PFs with 48-hour IC50s of 3.1 μM. and 1.6 μM, respectively. Altogether, these data are consistent with a model for *T. brucei* free fatty acid uptake mediated mainly by passive diffusion, followed by intracellular trapping by ACSs.

TT4G A Global Perspective on the Evolutionary Impetus for Glycosomes in Kinetoplastea

Hina Durrani (University of Minnesota); Sara L Zimmer (University of Minnesota)

The first steps of glycolysis are compartmentalized in kinetoplastids, to an organelle called the glycosome. A predominant hypothesis regarding the evolutionary benefit of glycosomes is that it facilitates efficient adaptation of the glycosomal metabolic repertoire. Turnover and replacement of glycosomes with those possessing a different enzyme composition is thought critical for sudden, major nutritional changes encountered throughout a kinetoplastid life cycle. This hypothesis is based mainly on *Trypanosoma brucei*, which can generate energy through cytochrome-mediated respiration in its Tsetse fly host but when replicating in a mammalian bloodstream generates energy only through glycolysis. Our objective is to establish the level of empirical support for this hypothesis across the Kinetoplastea. One approach is to determine the extent to which culturable kinetoplastids of various lifestyles execute a change their glycosomal enzyme composition in response to extracellular signals. We are initially pursuing glucose concentration as an extracellular signal, by modulating culture glucose for six kinetoplastids. Our selected species grow at different rates and to different maximal abundances, but all exhibited attenuated growth in low or no glucose. We measure abundances of likely-glycosomal enzymes in these species growing in consistent regular, low, and no glucose culture conditions, and during transition in concentration. When glucose is absent, at least some glycolysis enzymes appear less abundant in *T. brucei*, *Trypanosoma cruzi* and *Leptomonas pyrrhocoris*, but enzyme abundances of other species such as *Leptomonas barvae* appear largely insensitive to extracellular glucose concentration. Likewise, species range in sensitivity of enzyme abundance response to changes in glucose. Initial results suggest we revisit the origins and benefits of kinetoplastid glycosome enzyme compartmentalization.

Session V Abstracts – Gene Expression II

Pegine Walrad, Chair
Friday 8:45 AM-11:15 AM
September 16th 2022

5A Exploring the deepest secrets of the *Trypanosoma brucei* nuclear architecture

Claudia Rabuffo (LMU Munich); Anna Barcons-Simon (LMU Munich); Markus Schmidt (LMU Munich); T. Nicolai Siegel (LMU Munich)

In eukaryotes, gene expression is crucially linked to genome folding, which is tightly regulated by numerous factors, including regulatory DNA and RNAs. In bloodstream form trypanosomes, expression of the major antigen involves an inter-chromosomal contact bringing in spatial vicinity the active antigen gene and a major RNA maturation locus. Such functional genome organization has been uncovered using a chromosome conformation capture (Hi-C) method. Hi-C is based on quantifying frequencies of contacts between DNA loci and involves digestion of the genomic DNA with a restriction enzyme and ligation of DNA fragments to their closest neighbors. The restriction frequency poses a limit to the method resolution, which in turn curbs the identification of functional roles of genome folding. Here, we dramatically improved our architectural data resolution by establishing two methods that employ two restriction enzymes (Hi-C 3.0) or a micrococcal nuclease (Micro-C). Moreover, to further explore the complexity of the *T. brucei* nuclear architecture, we added an important piece of information to our analysis by establishing a method to map RNA-chromatin interactions (RADICL-seq). Finally, to analyze such complex datasets, we developed a software to interactively visualize, process and integrate them with other datasets. Together, these tools allowed us to identify new three-dimensional features of the *T. brucei* genome, such as loops bringing together transcription start sites. Our data suggest that in trypanosomes transcription might occur at discrete clusters, as shown for more complex eukaryotes. In mammals, transcription factories enable efficient transcription by concentrating RNA pol II and transcription factors in one place. Our data suggest that similarly, nuclear organization may contribute to gene expression regulation in African trypanosomes.

5B Site-saturation mutagenesis of core histone tail residues in *Trypanosoma brucei*

Markéta Novotná (University of Dundee); David Horn (University of Dundee)

It remains unclear to what extent and by which mechanisms transcription, DNA replication and DNA repair rely upon chromatin-based controls in the trypanosomatids. The N-terminal histone tails, and tail modifications, play key roles in these processes in other eukaryotes, although interpretation of writer, reader and eraser-defective phenotypes is complicated by potential perturbation of diverse and non-histone substrates. Genetic manipulation of core histones has also proven particularly challenging, because they are typically encoded by many identical genes. In the diploid trypanosomatids for example, approx. 40 copies of these genes are present in long arrays. The trypanosome N-terminal tails are also highly divergent relative to other model eukaryotes, suggesting potential novel mechanisms. We have used an CRISPR/Cas9 system to delete all native gene copies of histone H4 in *T. brucei*, complementing the defect with a single, recoded and highly expressed ectopic copy. These 'histH4one' strains were validated by genome sequencing and then inducible and templated CRISPR/Cas9 editing was used for site-saturation mutagenesis of N-terminal tail residues in the ectopic gene. Amplicon-seq profiling, used to monitor the relative fitness of these mutants, showed that substitution of lysine with arginine (non-acetylated mimic), K14R, was well-tolerated. Indeed, several amino acids were tolerated at this site (I, M, P for example), while aromatic residues had a significant negative impact on fitness. We are currently assessing saturation mutagenesis at H4-K10, acetylation of which is thought to impact transcription. Mutant pools and clones will be profiled for fitness, cell cycle control, DNA repair and gene expression phenotypes.

5C Structural Basis for Histone H3 Lysine 76 Dimethylation by *Trypanosoma brucei* DOT1A

Victoria Frisbie (Thomas Jefferson University); Hideharu Hashimoto (Thomas Jefferson University); Erik W Debler (Thomas Jefferson University)

Trypanosoma brucei has two highly homologous histone H3 lysine 76 (H3K76) methyltransferases TbDOT1A and TbDOT1B, which differ in their final methylation products (di- vs. trimethylated H3K76) and in function (cell cycle regulation vs. monoallelic VSG gene expression). For efficient methylation from me0 to me3, yeast and human DOT1 enzymes must transiently dissociate from the nucleosome to exchange the cofactor product AdoHcy for the methyl donor AdoMet. To avoid complete enzyme-substrate dissociation, interaction with monoubiquitin of histone H2B tethers yeast and human DOT1 enzymes onto the target nucleosome for consecutive reactions. By contrast, kinetoplastid DOT1 enzymes do not contain such a ubiquitin-binding region. Given that the majority of H3K76 in *T. brucei* cells is trimethylated, the mechanism of how TbDOT1A/B achieve higher methylation states without ubiquitin interaction remains elusive. Here we present crystal structures of TbDOT1A at 2.0 Å resolution to determine the mechanism of efficient H3K76 di- and trimethylation. We found that the active-site loop FH, which is involved in AdoMet binding and H3K76 substrate recognition, is flexible. This loop flexibility allows for AdoHyc/AdoMet exchange without dissociation from the target nucleosome, enabling TbDOT1A to efficiently methylate H3K76 from me0 to me2 in the absence of ubiquitin interactions. The sequence of the FH loop is highly conserved among kinetoplastid DOT1A and DOT1B, implicating a shared mechanism of these enzymes.

5D The telomere binding protein UMSBP2 is a chromatin remodeler that functions in regulation of gene expression and involved in antigenic variation in trypanosomes

Awakash Soni (Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School-Hebrew University); Olga Klebanov-Akopyan (Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School-Hebrew University); Esteban Erben (Universitat Heidelberg Zentrum fur Molekulare Biologie der Universitat Heidelberg University); Amnon Harel (The Azrieli Faculty of Medicine Bar-Ilan University); Katreena Yamin (The Azrieli Faculty of Medicine Bar-Ilan University); Itay Onn (The Azrieli Faculty of Medicine Bar-Ilan University); Joseph Shlomai (Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School-Hebrew University)

Packaging and condensation of genomes in the chromatin structure limit their accessibility to the transcription and replication machineries, while chromatin uncoiling by chromatin remodeling complexes renders the genome accessible to these activities. We studied the Universal Minicircle Sequence binding proteins (UMSBPs), which are CCHC-type zinc-finger proteins that bind the single-stranded G-rich UMS sequence, conserved at the replication origins of the mitochondrial DNA (kDNA) of trypanosomatids. *Trypanosoma brucei* nuclear UMSBP2, previously reported to function in the replication and segregation of kDNA, has been recently shown to colocalize with telomeres and play an essential role in chromosomal ends protection. Here, we report that UMSBP2 interacts selectively with core histones and linker histone H1 and that these interactions result in the remodeling of *T. brucei* chromatin. DNA remodeling by UMSBP2 is mediated via protein-protein interactions and is independent of its previously described DNA binding activity. UMSBP2 silencing, results in a higher nucleosomal density profile than in cells expressing TbUMSBP2 and this phenotype could be reversed by supplementing the depleted cells with UMSBP2. Depletion of UMSBP2 also results in telomeres deposition from the nuclear periphery to the nucleoplasm. UMSBP2 has the capacity to remodel chromatin structure in higher eukaryotes, as well as DNA condensed by a bacterial nucleoid-associated protein. RNA-seq analyses revealed that UMSBP2 knockdown affects the regulation of expression of multiple genes in *T. brucei*, with a most significant effect on the upregulation of the subtelomeric variant surface glycoproteins (VSG) genes, which mediate the antigenic variation process in African trypanosomes. These observations suggest that UMSBP2 is a chromatin remodeler that functions in the regulation of gene expression and plays an essential role in the control of antigenic variation in *T. brucei* cells.

5E Initiation of DNA replication in *Trypanosoma brucei* is regulated by the interaction of the RNaseH2 complex with DOT1 histone methyltransferases

Laura Hartleb (Department of Cell & Developmental Biology, Biocenter, University of Würzburg); Victoria Frisbie (Department of Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia); Hideharu Hashimoto (Department of Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia); Janna Kiselar (Department of Nutrition, School of Medicine, Case Western Reserve University, Cleveland); Falk Butter (Quantitative Proteomics, Institute of Molecular Biology (IMB), Mainz); Erik W. Debler (Department of Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia); Christian J. Janzen (Department of Cell & Developmental Biology, Biocenter, University of Würzburg)

Post-translational histone modifications such as lysine methylation influence the structure and the function of chromatin. DOT1 (disruptor of telomeric silencing 1) is a highly-conserved methyltransferase that exclusively methylates histone H3 lysine K79 (H3K79). *T. brucei* has two homologues, DOT1A and DOT1B, with differences in product specificity and function. While DOT1B mediates mono-, di-, and trimethylation of H3K76, DOT1A only catalyses mono- and di-methylation of this residue (H3K76me1/2). We showed previously that H3K76me1/2 is restricted to G2 phase and mitosis, and that DOT1A is a master regulator of replication initiation. RNAi-mediated depletion of DOT1A abolishes DNA replication completely whereas overexpression of DOT1A causes re-initiation of replication before the cell has completed S phase, implicating DOT1A as a master regulator of replication initiation. Since conserved regulatory domains are absent in these enzymes in *T. brucei*, the mechanism of cell cycle-dependent activity of DOT1 enzymes has remained elusive. Recently, we discovered that the ribonuclease H2 complex (RNaseH2) interacts with DOT1 enzymes in *T. brucei*. In vitro studies with recombinant enzymes and reconstituted nucleosomes demonstrated that RNaseH2 is an inhibitor of DOT1A activity. Using x-ray footprinting, we identified the interface region of the DOT1A/RNaseH2 complex and were able to abolish the inhibitory effect of RNaseH2 by introducing site-specific mutations into this site. Further experiments in vivo suggested that RNaseH2 interactions with DOT1 enzymes orchestrates their cell cycle-dependent activity in *T. brucei*, representing a novel mechanism of replication regulation.

5F RNase H1, a R-loop resolving enzyme, acts to suppress R-loop mediated DNA replication and limit genome variability in *Leishmania*

Jeziel Dener Damasceno (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Emma M. Briggs (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); João Reis-Cunha (Universidade Federal de Minas Gerais); Kathryn Crouch (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Craig Lapsley (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Daniella Bartholomeu (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology); Richard McCulloch (Univeristy of Glasgow - Wellcome Trust for Integrative Parasitology)

Stable RNA-DNA hybrids (R-loops) act in several genomic processes, but their roles in DNA replication are unclear. By using DRIP-seq, we show that R-loop distribution within each chromosome parallels the spatial, temporal and functional compartments of the unconventional DNA replication programme in *Leishmania*. Strikingly, R-loop levels correlate with chromosome size, which in turn correlate with replication timing. MFA-seq analyses shows that DiCre-mediated RNase H1 KO results in origin-independent DNA replication initiation, profoundly changing the DNA replication programme. Such alteration leads to genome-wide, chromosome-size dependent instability, including aneuploidy, SNPs and InDels, as revealed by whole genome sequencing. Also, ChIP-seq analyses shows that RNAse H1 is enriched at origins of DNA replication, suggesting that proper resolution of R-loops at those regions is essential for the homeostasis of genome duplication. Therefore, our data reveal a crucial role of RNase H1 in controlling R-loop-mediated DNA replication initiation, favouring conventional origin-directed initiation, and places the DNA-RNA hybrids as a pivotal player in *Leishmania* global genome plasticity.

5G New Insight Into kDNA Replication through Enzymatic Characterization of POLIB

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

Trypanosoma brucei has three mitochondrial DNA Polymerase I-like paralogs (POLIB, POLIC, POLID) that are independently essential to maintain the catenated kinetoplast DNA (kDNA) network. However, the precise division of labor among the paralogs, particularly which might be the replicative, proofreading enzyme remains enigmatic. De novo structure modeling of POLIB revealed a large insertion (369aa) in the polymerase thumb subdomain with homology to DEDDh DnaQ family 3'-5' exonucleases and a Glu to Ser substitution in Motif C typical for RNA polymerases that could favor ribonucleotide incorporation. These features are unique among Family-A DNA polymerases studied thus far and likely contribute to a specialized role in kDNA replication. We address this hypothesis by demonstrating the intrinsic enzymatic activities of POLIB. Here we demonstrate recombinant POLIB displays 3'-5' exonuclease degrading in a non-processive manner with DNA vs. RNA substrate preference. Exonuclease activity prevails over polymerase activity on DNA substrates at pH 8.0, while primer extension is favored at pH 6.0. Additionally, POLIB is able to extend an RNA primer more efficiently than a DNA primer in the presence of dNTPs but does not incorporate rNTPs efficiently. Mutations that ablate POLIB polymerase activity slow the exonuclease rate suggesting crosstalk between the domains. Immunoprecipitation of Pol I-like paralogs from *T. brucei* corroborates these findings including the preference for extension from an RNA primer. These data suggest POLIB is not the replicative polymerase for kDNA replication, but works cooperatively to facilitate extension by another paralog similar to the trio of specialized polymerases for canonical nuclear DNA replication.

TT5H An orphan, kinetoplastid-specific subunit of the Origin Recognition Complex?

Catarina Marques (University of Glasgow); Marcelo da Silva (University of Sao Paulo); Jose Batista (University of Glasgow); Calvin Tiengwe (Imperial College London); Richard McCulloch (University of Glasgow)

DNA replication is initiated at specific genomic loci termed origins, which are defined by the binding of a six-subunit Origin Recognition Complex (ORC). Though frequently described as highly conserved across eukaryotes, phylogenetic studies suggest many species may have lost one or more ORC subunits. In *Trypanosoma brucei*, four ORC subunits (ORC1/CDC6, ORC4, ORC5-like and ORC2-like) have been identified to date, all of which diverge considerably from their likely eukaryotic orthologues. The two remaining subunits, ORC3 and ORC6, remain elusive. Using a large group of publicly available genome and transcriptome datasets, we have run extensive sequence-based similarity searches, revealing Orc3 orthologues in all analysed Discoba lineages except for the kinetoplastids. Immunoprecipitation of multiple TbORC subunits resulted in the common identification of Tb1120 (Tb927.6.1120), and gel filtration analysis showed Tb1120 elutes in a complex of similar size to TbORC. Moreover, RNAi depletion resulted in growth arrest, cell cycle de-regulation, and abrogation of DNA replication. Sequence-based searches failed to provide evidence for a relationship between Tb1120 and any known ORC subunit, and orthologues of Tb1120 could not be found outside the kinetoplastids. However, de novo structure predictions suggest Tb1120 may have winged helix and AAA+ ATPase features found in ORC subunits. Our data suggest divergence of kinetoplastid ORC either through repurposing or replacement of at least one ORC subunit, an adaptation that has been accommodated by divergence of *T. brucei* ORC2 and ORC5 subunits. The potential purpose(s) of such restructuring of a core component of the DNA replication machinery remains unclear.

TT5l Mosaic VSGs Form as a Consequence of DNA Damage Repair in vitro and in vivo

Jaclyn E Smith (JHUSOM); Alex Beaver (JHUSOM); Shane Gilligan-Steinberg (JHBSPH); Sarah Sudlow (JHBSPH); Monica R Mugnier (JHBSPH)

Mosaic VSGs are crucial for *T. brucei* antigenic variation, the process where parasites change their surface antigen, a single VSG, to evade host antibody. There are ~2000 copies of VSG in the genome, consisting mostly of partial fragments and pseudogenes. Two or more VSGs, often incomplete themselves, combine to form novel VSGs known as mosaics. Mosaic VSG formation allows parasites to generate a nearly unlimited supply of new VSGs and is necessary for persistent chronic infection. Despite their importance, little is known about mosaic VSG formation or dynamics during infection. To this end, we developed VSG-Anchored Multiplex PCR Sequencing, a targeted sequencing approach to detect mosaic derivatives of a VSG of interest. Targeting VSG Antat1.1 in μMT mice, which lack B cells and never clear the initial AnTat1.1-expressing population, we observed patterns of recombination throughout AnTat, including numerous templated insertions consistent with DNA damage repaired through microhomology-mediated end joining (MMEJ). To investigate mosaic formation following DNA repair, we used an inducible Crispr/Cas9 system to introduce a double strand break within the N-terminus of AnTat1.1 in vitro. Remarkably, numerous AnTat1.1 mosaics formed immediately adjacent to the break site, with the same donor VSGs and patterns we observed in vivo. Accumulation of insertions over time, or insertions in particular positions, may be sufficient to produce novel antigens. These experiments demonstrate, for the first time, that mosaic VSGs can form as a consequence of DNA damage. Importantly, our observations and approach allow us to elucidate a previously inaccessible feature of antigenic variation.

TT5J A single pseudouridine on rRNA matters: A specific effect of rRNA modification on translation in *Trypanosoma brucei*

K. Shanmugha Rajan (Bar-Ilan University); Hava Madmoni (Bar-Ilan University); Saurav Aryal (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Beathrice Galili (Bar-Ilan University); Katherina Adler (Bar-Ilan university); Ron Unger (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Shulamit Michaeli (Bar-Ilan university)

Differential pseudouridine (Psi) modification on rRNA, snRNA, tRNA was suggested by us to play a role in the parasite transition between its two hosts (Chikne et al., Sci.Rep. 2016, Rajan et al., Nucleic Acids Res. 2019, Rajan et al., J. Biol. Chem. 2022). Here we used several mapping methods of pseudouridine such as Psi-seq, HydraPsiSeq and nanopore RNA sequencing to quantitatively determine changes in pseudouridine on rRNA of procyclics (PCF) and bloodstream (BSF) parasites. The hypermodified sites in BSF were localized to the H69 domain present in the inter-subunit bridge, A-site finger domain, and near the decoding center. To investigate the role of these hypermodified sites and their implications, we performed CRISPR-Cas9 knock-out of the snoRNA TB11Cs6H1 guiding Psi-530 on H69. The growth of sKO was compromised and quantitative mass-spectrometry identified changes in the level of only a subset of proteins; most are developmentally regulated. Add back of TB11Cs6H1, but not a defective mutant was able to reverse the sKO phenotype. To investigate the mechanism by which a single modification affects translation, rRNA structure was determined by dimethyl sulfate (DMS) mapping in vivo and changes in the structure of H69 and other rRNA domains, were observed. Analysis of 80S ribosomal protein composition detected major reduction the level of eS12. We propose that the change in Psi-530 affected the secondary structure of the rRNA, leading to dislodging of eS12 and producing ribosomes depleted of this protein. These “altered” ribosomes result in specific changes in translation. Thus, the differential level of Psi modification in the two life stages may contribute to stage-specific translation regulation during cycling between the hosts.

Session VI Abstracts – RNA Editing

Laurie Read, Chair
Friday 2 PM-4:10 PM
September 16th 2022

6A RNA Editing: Defining Trait of Kinetoplastids

Kenneth D. Stuart (Center of Global Infectious Disease Research, Seattle Children's Research Institute)

RNA editing is the intramitochondrial post transcriptional U insertion and deletion process that generates functional mRNAs that are encoded in the mitochondrial DNA, i.e. kinetoplast DNA (kDNA), for which Kinetoplastids are named. Transcripts from kDNA maxicircles are precisely edited as specified by guide RNAs (gRNAs) almost all of which are transcribed from the very numerous kDNA minicircles. The editing performs hundreds of precise U insertions and deletions to generate the translatable mRNAs. This employs the functions of roughly 100 nuclear encoded cytoplasm translated proteins many of which are organized in multiprotein complexes. These include: (1) the Catalytic Complexes (CCs) that perform three-step cycles of mRNA cleavage, U addition or removal, and ligation at each site of editing; (2) Substrate Complexes (SCs) that associate with mRNAs, gRNAs and CCs; (3) complexes that process the ends of and interactions between gRNAs and mRNAs. These complexes and other proteins function in concert to edit multiple blocks of several RNA editing sites to generate translatable mRNAs. The abundances of various edited mRNAs, which encode oxidative phosphorylation (ox-phos) system components, differ between life cycle stages in *T. brucei* and correlate with energy production by oxphos in procyclic forms vs glycolysis in bloodstream forms. Expression of these edited mRNAs is coordinated with other processes that differ between the life cycle stages, including those for extramitochondrial processes, e.g. surface protein gene expression. Aspects of editing differs among the various kinetoplastids which likely reflects their metabolic and life cycle differences. Kinetoplastid RNA editing shares features with other types of nucleic acid processing, e.g. RNA splicing, and its elucidation may lead to practical applications.

6B Novel Roles of REH2C Helicase Complex in Editing Fidelity and as RNA-Remodeler in two life-cycle stages of *Trypanosoma brucei*

Joshua Meehan (Texas A&M University); Suzanne McDermott (Seattle Children's Research Institution); Al Ivens (University of Edinburgh); Zachary Goodall (Texas A&M University); Achim Schnaufer (University of Edinburgh); Jia Woo (Harvard Medical School); Silvi Rouskin (Harvard Medical School); Zihao Yu (Texas A&M University); Lanying Zeng (Texas A&M University); Xiren Zhang (Texas A&M University); Jorge Cruz-Reyes (Texas A&M University)

U-indel RNA editing of the mitochondrial mRNAome in *T. brucei* is differentially controlled in bloodstream and procyclic forms by mechanisms that remain fundamentally unknown. The U-indels create translatable transcripts and are directed by antisense guide RNAs (gRNAs) in the “editosome” holoenzyme. Editosomes are dynamic and include several factors including REH2C (RNA Editing KREH2-Associated Complex) which we identified and showed, via RNA-Seq, that it controls the total number of edits and their fidelity in procyclics (Kumar et al., JBC 2016 & RNA 2020). The core DExH/RHA RNA Helicase (KREH2) subunit in REH2C exhibits 3’-5’ ATP-dependent unwinding activity and binds RNA. We have now identified a novel role of the REH2C machine as “RNA remodeler”. We adapted DMS-MaPseq technology (DMS-probing coupled with RNA-Seq) in trypanosome editing to show that REH2C loss-of-function dramatically changes RNA secondary-structure. The newly identified RNA structures prevents progression of early editing and their formation involves editing fidelity changes under REH2C control. Notably, such structures are used differently during development in mRNAs A6 (edited in both stages) and ND7 (repressed editing in procyclics): In A6, REH2C prevents a “repressive” structure. In contrast, in ND7, REH2C naturally promotes a “repressive” structure in procyclics. However, REH2C loss-of-function prevents this structural block and “artificially” increases editing fidelity in ND7. In summary, the REH2C complex exhibits a novel role as an RNA remodeler in differential editing. Finally, we examined the localization of REH2C and other editing complexes, in WT and mutant parasites in mitochondria using immunofluorescence and super-resolution microscopy.

6C KREH1 RNA helicase activity promotes utilization of initiator gRNAs across multiple mRNAs in trypanosome RNA editing

Ashutosh P Dubey (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Brianna L Tylec (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Amartya Mishra (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Runpu Chen (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Yijun Sun (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo); Laurie K Read (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo)

In *Trypanosoma brucei*, 12 out of 18 mitochondrially encoded mRNAs require post-transcriptional insertion and deletion of uridine (U) residues to generate translatable mRNAs. Editing is essential, as these mRNAs primarily encode components of the mitochondrial respiratory system. U insertion/deletion RNA editing is directed by trans-acting gRNAs and mediated by holoenzymes comprising three protein complexes: the catalytic RNA Editing Core Complexes (RECC), RNA Editing Helicase 2 Complex (REH2C), and the non-catalytic RNA Editing Substrate Binding Complex (RESC). Another ATP-dependent RNA helicase, Kinetoplast RNA Editing Helicase 1 (KREH1), interacts transiently with the holoenzyme, although its function is poorly understood. Previous evidence indicates a role for KREH1 in the editing of at least some mRNAs, with a suggestion that it functions in gRNA removal. Here, we show that KREH1 knockout (KO) impairs editing of a subset of mRNAs, and cells overexpressing helicase-dead mutants exhibit expanded impairment of editing across multiple transcripts. Quantitative RT-PCR and high throughput transcriptome analysis reveals impaired editing initiation and progression in KREH1 KO and mutant-expressing cells. Strikingly, these cells exhibit a distinct defect in the earliest stages of editing in which the initiator gRNA is bypassed, and small number of editing events takes place just outside this region. Overexpressed wild type KREH1 and mutant KREH1 interact similarly with RNA and holoenzyme, and overexpression of both similarly disorders holoenzyme homeostasis. Thus, our data support a model in which KREH1 RNA helicase activity facilitates remodeling of initiator gRNA-mRNA duplexes to permit accurate utilization of initiating gRNAs on multiple transcripts.

6D Structure of RNA Editing Substrate Binding Complex

Ruslan Afasizhev (Boston University); Shiheng Liu (UCLA); Inna Afasizheva (Boston University); Hong Wang (Boston University); Ana Lucia Alvarez-Cabrera (UCLA); Xiaorun Li (UCLA); Jane Lee (UCLA); Jason Hu (UCLA); Zihang Li (UCLA); Clinton Yu (UC Irvine); Lan Huang (UC Irvine); Fan Zhang (Shanghai Tech); Liye Zhang (Shanghai Tech); Z. Hong Zhou (UCLA)

Discovery of post-transcriptional U-insertions and deletions in mitochondrial mRNAs of *Trypanosoma brucei* introduced the term “RNA editing” and led to guide RNA concept. Trypanosomes possess the kinetoplast, a mitochondrial nucleoprotein body that encloses interlinked maxicircles and minicircle DNAs. The maxicircle encodes 9S and 12S rRNAs, six protein genes and 12 cryptogenes, while minicircles supply more than 900 gRNAs. To become a translation-competent mRNA, cryptic transcript undergoes 5′ modification, 3′-5′ exonucleolytic trimming, 3′ adenylation, massive internal U-insertion/deletion editing, and, lastly, 3′ A/U-tailing. RNA editing substate binding (RESC), RNA editing catalytic (RECC) and remodeling (REH2C) complexes assemble into the “editosome” to carry out gRNA-programmed editing. Here, we report the first high-resolution structures of an editing complex. Cryo-EM study of RNA editing substate binding complex identified isoforms RESC-A and RESC-B, which share two subunits but otherwise are distinct. Six-polypeptide RESC-A binds hairpin-folded gRNA via RESC1/2 heterodimer in a conformation that precludes gRNA-mRNA hybridization. We observe a remarkable structural homology between RESC2 and Cet1 RNA triphosphatase and definitively place gRNA’s triphosphorylated 5′ end into RESC2 active site. Structure of the nine-polypeptide RESC-B particle shows gRNA in an extended conformation proximal to mRNA and reveals mRNA binding path through the entire complex. In combination with biochemical and genetic data, we will present a model of RESC-A to RESC-B transition and discuss mechanistic implications of these findings for the molecular mechanism of mitochondrial RNA editing.

6E Domain function and structure of three heterodimeric endonuclease subunits in RNA editing catalytic complexes

Suzanne McDermott (Seattle Childrens Research Institute); Jason Carnes (Seattle Childrens Research Institute); Isaac Lewis (Seattle Childrens Research Institute); Maxwell Tracy (Seattle Childrens Research Institute); Ken Stuart (Seattle Childrens Research Institute)

RNA editing in trypanosomes involves post-transcriptional U insertion and deletion to create functional mitochondrial mRNAs. Multiple multiprotein complexes are required for editing at numerous sites using template guide RNAs (gRNAs). These include three related multienzyme complexes called RNA Editing Catalytic Complexes (RECCs) that catalyze RNA cleavage, U insertion or deletion, and ligation. Each RECC has a distinct endonuclease activity that requires two related RNase III proteins, with only one competent for catalysis. We identified multiple loss-of-function mutations in the RNase III and other motifs of the non-catalytic KREPB6, KREPB7, and KREPB8 endonuclease components by random mutagenesis and screening. These mutations had various effects on growth, editing, and both the abundances and RECC associations of these RNase III protein pairs in bloodstream form (BF) and procyclic form (PF) cells. Protein structure modelling predicted that the Zinc Finger (ZnF) of each paired RNase III protein contacts RNA positioned at the heterodimeric active site which is flanked by helices of a novel RNase III-Associated Motif (RAM). The results indicate that the protein domains of the non-catalytic subunits function together and with those of the catalytic subunits in RECC integrity, substrate binding, and editing site recognition during the multistep RNA editing process. Additionally, several mutants display distinct functional consequences in different life cycle stages. Overall, these results highlight the complementary roles of the non-catalytic/catalytic protein pairs that make up the endonucleases within the three *T. brucei* RECCs that mature mitochondrial mRNAs.

6F TUTase-Exonuclease-RNA Helicase Nexus Governs Mitochondrial RNA Biogenesis in Trypanosomes

Inna Afasizheva (Boston University); Takuma Suematsu (Boston University); Andres Vacas (Boston University); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine)

Discoveries of terminal RNA uridyltransferases (TUTases) in mitochondria of trypanosomes and, subsequently, in most eukaryotes positioned 3′ uridylation as the major epitranscriptomic factor. From RNA editing to maternal mRNA clearance, the TUTase catalytic domain has acquired divergent auxiliary modules that determine RNA specificity and complex incorporation. We have identified the mitochondrial 3′ processome (MPsome) composed of KRET1 TUTase, KDSS1 3′-5′ exonuclease, and six structural proteins. In this complex, TUTase activates the exonuclease to process all classes of primary transcripts and to degrade mature RNAs. We find that compositionally distinct MPsome isoforms execute RNA class-specific transactions, which involve double-stranded RNA intermediates arising from cryptic antisense molecules. These duplexes block MPsome's progression along a precursor thus generating mature RNAs. Contribution of an unknown factor to resolving ds-intermediates has been hypothesized but its identity remained unknown. Here, we report a novel DEAD-box RNA helicase, termed KREH3, that fulfils a key mechanistic step: upon MPsome stalling near the ds-region, the MPsome-associated KREH3 unwinds the duplex, and binds to and stabilizes properly trimmed RNAs. We demonstrate that KREH3's ATP-dependent activity is essential for processing rRNA, mRNA and gRNA precursors. We further show that TUTase, exonuclease, helicase, and poly(A) polymerase co-localize in antipodal puncta adherent to the kinetoplast. Conversely, the RNA editing machinery is distributed diffusely in the matrix while the ribosomes assemble on inner membrane. Collectively, our findings identify a critical element of kRNA processing and reveal that spatially and functionally coupled TUTase, 3′-5′ exonuclease and ATP-dependent remodeling activities underpin mitochondrial RNA biogenesis in Trypanosomes.

Session VII Abstracts – Antigenic Variation

Christian Janzen, Chair
Saturday 9 AM-11:00 AM
September 17th 2022

7A Extravascular spaces are reservoirs of antigenic diversity in *Trypanosoma brucei*

Alexander Beaver (Johns Hopkins School of Medicine); Nathan P Crilly (Johns Hopkins School of Medicine); Bailin Zhang (Johns Hopkins Bloomberg School of Public Health); Bryce Bobb (Johns Hopkins Bloomberg School of Public Health); Filipa Rijo-Ferreira (Berkeley Public Health Molecular and Cell Biology Department); Luísa M Figueiredo (Universidade de Lisboa); Monica Mugnier (Johns Hopkins Bloomberg School of Public Health)

Trypanosoma brucei lives an entirely extracellular life cycle in its mammalian host, facing a constant onslaught of host antibodies. The parasite evades clearance by the host immune system through antigenic variation of its dense variant surface glycoprotein (VSG) coat, periodically “switching” expression of the VSG using a large genomic repertoire of VSG-encoding genes. Studies of antigenic variation in vivo have focused exclusively on parasites in the bloodstream, but recent work has shown that most parasites are extravascular and reside in the interstitial spaces of tissues. However, it is unknown whether parasites undergo antigenic variation while in extravascular spaces. We sought to explore the dynamics of antigenic variation in extravascular parasite populations using VSG-seq, a high-throughput sequencing approach for profiling VSGs expressed in populations of *T. brucei*. Our experiments show that the expressed VSG repertoire is not uniform across populations of parasites within the same infection and that a greater number of VSGs are expressed in tissue spaces than in the blood. More than 75% of the VSGs detected in an animal were found exclusively within extravascular spaces. Interestingly, we also noticed a delay in the VSG-specific clearance of parasites in tissue spaces compared to the blood. This finding aligns with a model in which parasites “hide” from the immune system in tissue spaces, where a slower immune response provides them with more time to generate new antigenic variants. Overall, our results show that extravascular spaces are significant reservoirs of VSG diversity, potentially resulting from delayed clearance of tissue-resident parasites.

7B Allosteric regulation of repressor-activator protein 1 by PI(3,4,5)P3 controls on and off switching of telomeric expression sites in trypanosomes

Abdoulie O. Touray (McGill University); Rishi Rajesh (McGill University); Tony Isebe (McGill University); Mira Loock (McGill University); Oksana Kutova (McGill University); Igor Cestari (McGill University)

Trypanosoma brucei evades the host antibody clearance by switching its variant surface glycoprotein (VSG) coat. *T. brucei* has over 2,000 VSG genes; however, only one VSG is transcribed at a time from one of the 20 telomeric expression sites (ESs). Antigenic variation occurs via transcriptional switching between ESs or VSG gene recombination. We show that VSG monogenic expression and switching involve a nuclear phosphatidylinositol phosphate 5-phosphatase (PIP5Pase) enzyme and its binding partner repressor-activator protein 1 (RAP1). PIP5Pase catalytic mutation (D360A/N362A) inhibiting PI(3,4,5)P3 dephosphorylation results in transcription of all silent telomeric VSG genes in bloodstream or procyclics and increases VSG switching. ChIP-seq analysis with RAP1 showed that it binds ES sequences flanking VSG genes, namely 70 bp and telomeric repeats. Using gel shift and binding kinetic assays, we found that RAP1 C-terminal Myb or Myb-like domains bind directly to 70 bp or telomeric repeats. In contrast, we found that RAP1 N-terminus has a villin headpiece domain and binds to PI(3,4,5)P3. Notably, PI(3,4,5)P3 disrupted RAP1 association with telomeric or 70 bp repeats in vitro. To investigate how PI(3,4,5)P3 regulates RAP1 association with ESs in vivo, we performed ChIP-seq with RAP1-HA in *T. brucei* cells exclusively expressing the wildtype or catalytic mutant PIP5Pase. PIP5Pase inactivation abolished RAP1 association with telomeric and 70 bp repeats and resulted in VSG transcription. We propose that PI(3,4,5)P3 is an allosteric regulator of RAP1 and controls its association with telomeric ESs, thus providing an on/off switch mechanism for ES transcriptional activation and silencing during antigenic variation and parasite development.

7C Multi-allelic exclusion by an allele-selective RNA-DNA helicase in African trypanosomes

Joana RC Faria (York Biomedical Research Institute, Department of Biology, University of York, UK); Hideharu Hashimoto (Thomas Jefferson University, USA); Michele Tinti (Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, UK); Catarina A Marques (Wellcome Trust Centre for Integrative Parasitology, University of Glasgow, UK); Erik W Debler (Thomas Jefferson University, USA); David Horn (University of Dundee, UK)

Large gene-families often exhibit monogenic expression, with contingent processes including antigenic variation for immune evasion in parasites, and olfaction and B-cell development in mammals. Despite intense study, the mechanisms that underpin these paradigmatic examples of stochastic gene choice and exclusion remain somewhat mysterious. Substantial recent progress yielded Variant-Surface-Glycoprotein (VSG) exclusion factors in African trypanosomes and a new appreciation of the context in which they operate. Specifically, VSG-exclusion-2 (VEX2) accumulates at the active-VSG expression-site and binds VEX1 at a trans-splicing locus on another chromosome; other VSGs are excluded from this sub-nuclear expression-factory (PMIDs: 31289266; 33432154). We performed single-cell-RNA-Seq following VEX2 depletion, revealing a surprisingly complex mixture of simultaneously active VSGs in individual trypanosomes. Notably, we also observed a consistent hierarchy of VSG derepression. ChIP-Seq indicated strong enrichment of VEX2, which forms a native complex of ~1 MDa, at the active-VSG expression-site. Using super-resolution microscopy, VEX2 N- and C-termini were distinctively visualised extending towards the active-VSG and the splicing locus, respectively, revealing an allele-selective inter-chromosomal bridge. Further, a recombinant predicted helicase core from VEX2 displayed RNA-DNA-helicase activity in vitro. To assess whether this activity was required for VSG-exclusion, we established a CRISPR/Cas9-mediated saturation-mutagenesis assay using FACS followed by amplicon-Seq profiling. Replacement of a critical amino acid with any other amino acid disrupted allelic exclusion. Finally, we found that the VEX1 N-terminus interacts with VEX2, stabilising it and sustaining exclusion. This work begins to reveal the mechanisms by which the VEX complex promotes stochastic VSG gene choice and allelic exclusion in African trypanosomes.

7D Heterogeneous elongation of RNA polymerase I transcription at the active VSG expression site in *Trypanosoma brucei*

James Budzak (Imperial College London); Gloria Rudenko (Imperial College London)

A Variant Surface Glycoprotein (VSG) coat protects bloodstream form *Trypanosoma brucei* within the mammalian host. VSG is the most abundant mRNA in the cell, with the active VSG gene expressed from one of ~15 VSG expression sites (ES). The active ES is transcribed by RNA Polymerase I (Pol I) in a unique extra-nucleolar Expression Site Body (ESB). *T. brucei* must maintain very high levels of VSG expression, and it has therefore been assumed that the active ES is always fully transcribed. However, using different microscopy approaches we have consistently observed that transcription of the active ES may be heterogeneous. We investigated this further using cells in which MS2 sequences were inserted at 2, 10, 20, 40 or 60 kb downstream of the active ES promoter. Using single molecule RNA-FISH experiments, we now show that Pol I transcription elongation is not always fully processive at the active ES. We find that transcription elongation is gradually attenuated at the active ES with the highest pre-mRNA levels 2kb downstream of the active ES promoter, and the lowest pre-mRNA levels 60kb downstream, immediately upstream of the telomeric VSG. Interestingly, we find a significant reduction in transcription after the first ~10kb of the active ES. Despite this decrease, pre-mRNA levels near the active ES telomere are still higher than levels at Pol II transcribed loci. These unexpected observations challenge the paradigm that Pol I provides continuously high levels of transcription throughout the active ES in *T. brucei*.

7E The RNA Recognition Motif (RRM)-mediated RNA binding activity in *T. brucei* RAP1 is essential for VSG monoallelic expression

Amit Kumar Gaurav (Cleveland State University); Marjia Afrin (Cleveland State University); Xian Yang (The Hong Kong Polytechnic University); Arpita Saha (Cleveland State University); Xuehua Pan (The Hong Kong Polytechnic University); Zeyang Ji (Hong Kong University of Science and Technology); Kam-Bo Wang (The Chinese University of Hong Kong); Mingjie Zhang (Hong Kong University of Science and Technology); Yanxiang Zhao (The Hong Kong Polytechnic University); Bibo Li (Cleveland State University)

Trypanosoma brucei, a protozoan parasite causing human African trypanosomiasis, sequentially expresses immunologically distinct VSGs, its major surface antigen, from subtelomeric loci in a monoallelic manner to evade the host immune response. We have shown that the telomere protein TbRAP1's dsDNA binding activity is essential for silencing VSGs globally, but how TbRAP1 permits the active VSG expression is unknown. Unexpectedly, we identify an RNA Recognition Motif (RRM) in TbRAP1 through NMR structural analysis and an RRM-mediated RNA binding activity in TbRAP1 through both in vitro and in vivo analyses. This is a novel finding, as none of known RAP1 homologs has any RRM domain or RNA binding activity. TbRAP1 RRM recognizes VSG 3'UTRs in vitro and binds the active VSG RNA in vivo. Mutating key RRM residues abolishes the RNA binding activity, significantly decreases the active VSG RNA level and derepresses silent VSGs. The TbRAP1's RNA and dsDNA binding activities are mutually exclusive and compete with each other in a concentration-dependent manner, suggesting a novel mechanism of monoallelic expression where abundant RNA from the active VSG antagonizes TbRAP1's dsDNA binding-mediated silencing effect locally to sustain the full-level VSG transcription.

7F *Trypanosoma brucei* RNA/DNA hybrid interactomes reveal a role for RAD51 in R-loop homeostasis and repair of VSG-localised DNA breaks during antigenic variation

Mark Girasol (University of Glasgow); Emma Briggs (University of Edinburgh); Jeziel Damasceno (University of Glasgow); Catarina Marques (University of Glasgow); Marija Krasilnikova (University of Glasgow); Craig Lapsley (University of Glasgow); Ross Carruthers (University of Glasgow); Dario Beraldi (University of Glasgow); Pilarita Rivera (University of the Philippines); Richard McCulloch (University of Glasgow)

R-loops are three-stranded nucleic acid structures comprised of an RNA/DNA hybrid in the context of a displaced single-stranded DNA and usually arise when an elongating transcript reinvades the template DNA, but can also occur in trans. R-loops are involved in many crucial processes in all organisms and are therefore under tight regulatory control. Mapping R-loops in the *Trypanosoma brucei* genome revealed widespread enrichment, including in subtelomeric Variant Surface Glycoprotein (VSG) expression sites, linking them to DNA damage and antigenic variation. Using RNA/DNA hybrid immunoprecipitation coupled with mass spectrometry, 463 and 351 putative R-loop–interacting proteins were identified in bloodstream and procyclic form parasites, respectively, including activities linked to RNA processing and DNA replication and repair. Amongst putative interactors that appeared more enriched in bloodstream cells were two recombination factors with known roles in VSG switching, RAD51 and RAD51-3, and two other proteins with unreported functions, a putative SNF2 chromatin remodeler and an ATP-dependent DEAD/H RNA helicase (DH). Loss of each protein led to nuclear genome damage and alterations in VSG expression dynamics. RNA/DNA hybrid immunofluorescence analysis revealed that loss of RAD51 resulted in a global decrease in R-loop abundance, while depletion of SNF2, DH, or RAD51-3 led to a global increase. Genome-wide mapping of R-loop distribution in RAD51 mutants indicated depletion in R-loops at multiple genomic sites, including VSG-associated 70-bp repeats. Moreover, using Breaks Labelling In Situ and Sequencing (BLISS) we found pronounced levels of DNA breaks that localise to the 3’ end of the expressed VSG and become more abundant in RAD51 mutants. Our data reveal multiple unexplored activities that may influence R-loop function in the *T. brucei* genome, and suggest R-loops form, via RAD51, around DNA breaks at the expressed VSG during antigenic variation.

Poster Session A Abstracts

Wednesday 7 PM
September 14th 2022

1 TT1G The role of ESCRT in endocytosis and secretion of extracellular vesicles trypanosomatids

Nadjania S de L Silva (Unifesp); Suellen R Maran (Unifesp); Nilmar S Moretti (Unifesp); Ana C Torrecilhas (Unifesp); Sergio Schenkman (Unifesp)

The ESCRT machinery, consists of four multi-subunit complexes, ESCRT-0 to IV involved in the transport of cargoes to form multivesicular bodies in cell endosomal compartments. These endosomes fuse with lysosomes for cargo degradation or with the plasma membrane to produce extracellular vesicles. Here, we studied the role of the ESCRT-I complex in *Trypanosoma cruzi* and *Leishmania mexicana*. Parasites expressing Cas9 and T7RNA polymerase were transfected with DNA fragments coding for two sgRNA to cleave the coding sequence ends of the VPS23 gene, a member of the ESCRT-I, together with DNA donors coding for hygromycin and/or blasticidin resistance. Only one of the VPS23 alleles could be replaced, while several other double knockouts were obtained, suggesting that VPS23 is essential for *T. cruzi* and *L. mexicana*. Nevertheless, these partial knockouts significantly lower the receptor mediated endocytosis of transferrin, but not the fluid phase BSA-uptake in *T. cruzi*. In addition, they reduced the secretion of extracellular vesicles by *T. cruzi* epimastigotes. The partial knockout did not affect proliferation and metacyclogenesis, and the resulting *T. cruzi* trypomastigotes were able to infect cells as the parental cell lines. We are currently analyzing whether the secretion of extracellular vesicles is also changed in *T. cruzi* trypomastigotes released from infected mammalian cells and the capacity of these parasites to promote infection. These results suggested an essential role of TcVPS23 by modulating endocytic and secretory activities in both *T. cruzi* and *L. mexicana*.

2 TT1H Spatial regulation of the multifunctional dynamin-1-like protein in *Trypanosoma brucei*

Madeline F Malfara (Villanova University); Alexa Pereira (Villanova University); Megan L Povelones (Villanova University)

Trypanosoma brucei undergoes morphological changes to adapt to host environments, including remodeling of their mitochondrial network. In yeast and mammals, membrane remodeling events involve both classical dynamins and dynamin-related proteins (DRPs), with DRPs mediating organelle division. In contrast, *T. brucei* have only one multifunctional dynamin-like protein (TbDLP) with roles in endocytosis and mitochondrial fission. Currently there are no known interacting proteins for TbDLP, and uncovering its molecular mechanism remains complicated by its involvement in multiple cellular pathways. Using tandem affinity purification in the related kinetoplastid *Crithidia fasciculata*, we have identified 30 putative DLP interacting proteins, almost a third of which have been found associated with glycosomes, glycolytic organelles related to peroxisomes. To explore the role of TbDLP in glycosome structure, we localized a glycosomal marker in procyclic form *T. brucei* during TbDLP RNAi and overexpression. While control and TbDLP knockdown cells showed a granular pattern, overexpression of TbDLP caused an increase in cytoplasmic signal, indicating a defect in glycosomal import. Intriguingly, overexpression of TbDLP also alters its cellular localization, changing from cytoplasmic puncta to accumulations in the periflagellar pocket region. When we probed *T. brucei* lysates for endogenous TbDLP by western blot, we found two bands of similar size, possibly representing modified forms. While both bands decrease during RNAi, overexpression of TbDLP increases only the lower band. We favor a hypothesis whereby overexpression of TbDLP alters its post-translational modification and therefore its subcellular localization, providing a possible mechanism for the spatial regulation of TbDLP function.

3 TT2E A quantitative mass spectrometry approach to identify novel proteins involved in macrophage infections by *Leishmania* parasites

Nicolas Hagedorn (Department of Cell and Developmental Biology, Würzburg, Germany); Albert Fradera Sola (Institue of Molecular Biology, Mainz); Elisa Theres Rauh (Department of Cell and Developmental Biology, Würzburg, Germany); Tobias Gold (Microbiology Institute-Clinical Microbiology, Erlangen); Ulrike Schleicher (Microbiology Institute-Clinical Microbiology, Erlangen); Falk Butter (Institue of Molecular Biology, Mainz); Christian J. Janzen (Department of Cell and Developmental Biology, Würzburg, Germany)

Leishmania are single-celled parasites with an intracellular life cycle stage in phagocytic cells of their vertebrate hosts. Several transcriptomic analyses of host cell defense mechanisms and how the parasites respond to these processes are already available. Due to the prevalence of post-transcriptional regulation in kinetoplastids however, transcriptomes do not reflect the protein composition very well. Here, we present a systematic comparative in vitro study of the infection process using a quantitative proteomics approach. Murine bone marrow-derived macrophages (BMDMs) were infected with 3 different *Leishmania* species (L. mexicana, *L. major*, *L. infantum*). Whole cell protein extracts of parasites and BMDMs were prepared at 7 different timepoints post-infection (0.5-72 h) and were analysed using quantitative mass spectrometry. We were able to quantify the expression of approximately 2000 murine proteins in all 3 infection experiments, while 1500, 1000 and 1400 proteins were analysed after infection with L. mexicana, *L. major* and *L. infantum*, respectively. The number of differentially-expressed proteins during the time course of infection varied for each species (349, 133, and 287 proteins for L. mexicana, *L. major* and *L. infantum*, respectively). Our experiments identified many proteins putatively involved in the infection process, which were not found in previously published transcriptome analyses. For example, gene ontology enrichment showed 41 L. mexicana protein IDs involved in oxidoreductase activity. Additionally, we found 24 differentially-expressed proteins without any functional or structural annotation in the TriTryp database. We are currently investigating several candidate proteins to discover novel mechanisms involved in parasite adaptation processes during macrophage infections.

4 A human adipose tissue model for *Trypanosoma brucei* infection

Laura Hauf (Universität Würzburg); Carina Blum (Universitätsklinikum Würzburg); Torsten Blunk (Universitätsklinikum Würzburg); Markus Engstler (Universität Würzburg)

Despite awareness of *Trypanosoma brucei* colonizing multiple host tissues, biological mechanisms underlying parasite life within extravascular spaces remain unresolved. One tissue that is vital to our understanding of this parasite is the skin. At the site of transmission, the interface between host, parasite, and the tsetse fly vector is created. To investigate African trypanosomes within host skin, advanced primary human skin equivalents were developed, comprising an epidermal and dermal layer. These human skin equivalents were utilized to characterize parasite differentiation and persistence following natural infection vs. syringe transmission. Taking yet another step forward, we are aiming to extend our model by incorporating the hypodermis as the most interior skin layer. Based on a previously described adipose tissue construct, we are establishing an infection system to monitor *T. brucei* inside the fat. For the preparation of fat-like tissue layers, spheroids are generated from human adipose-derived stromal cells (ASC) and seeded into fibrous scaffolds produced by melt electrowriting (MEW). Subsequently, ASCs within the tissue sheets are differentiated into the adipogenic lineage. So far, the artificial adipose tissue model was successfully co-cultured with bloodstream-form cells as well as infected with vector-borne metacyclic forms of *T. brucei*. With this, we now have the tools to combine the fat tissue component and human skin equivalents in a sandwich approach and address complex questions regarding metabolism and tissue tropism of the parasites.

5 TT2F Trypanosome sequestration and immune response drive acute cerebral disease

Sara Silva Pereira (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Mariana De Niz (Institut Pasteur); Karine Serre (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Joana Coelho (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Marie Ouarné (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa "); Cláudio A Franco (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Luísa M Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

Trypanosoma congolense causes different pathologies in animals, in Africa. Cerebral trypanosomiasis is an acute form of disease, but the mechanism underlying its severity remains unknown. Using a multidisciplinary approach, we developed a mouse model of acute cerebral trypanosomiasis and characterized the cellular, behavioral and physiological consequences of this infection. By intravital microscopy, we showed that *T. congolense* 1/148 strain parasites heavily sequester in the brain vasculature for long periods of time (up to 8 hours). Endothelial-specific ribosome profiling showed that parasite presence results in a strong pro-inflammatory response. We further observed that mice infected with this strain displayed significant and quantifiable neurological clinical signs, which were associated with an overwhelming ICAM1-mediated, CD4+ T cell extravasation into the brain parenchyma. Antibody-mediated ICAM1 blocking and lymphocyte absence reduced parasite sequestration in the brain and prevented the onset of cerebral trypanosomiasis. Furthermore, adoptive cell transfer experiments showed that CD4+ T cells, alone, are sufficient to cause the neuropathology that culminated in mouse death. We propose a mechanism whereby acute cerebral trypanosomiasis depends both on parasite (sequestration) and host factors (ICAM1 and CD4+ T cells). This is a new experimental model that adds novel tools to understanding trypanosomiasis. It will allow researchers to understand the variety of different clinical outcomes in *T. congolense* infections.

6 TT2GDevelopment of a molecular diagnostic toolbox for the detection of trypanosomatid parasites by SHERLOCK

Elena Pérez-Antón (Institut Pasteur, Université Paris Cité); Roger Junior Eloiflin (Institut de Recherche pour le Développement (IRD)-CIRAD); Annick Dujeancourt-Henry (Institut Pasteur, Université Paris Cité); Aïssata Camara (Institut Pasteur of Guinea); Jean-Mathieu Bart (Institut de Recherche pour le Développement (IRD)-CIRAD); Brice Rotureau (Institut Pasteur, Université Paris Sorbonne; and Institut Pasteur of Guinea); Lucy Glover (Institut Pasteur, Université Paris Cité)

The availability of highly sensitive and specific diagnostic tools, adapted to point-of-care, is crucial for the successful control of infections caused by trypanosomatid parasites. We have adapted SHERLOCK (for Specific High-sensitivity Enzymatic Reporter unLOCKing (Kellner et al. 2019; PMID: 31548639) for the rapid and specific detection of trypanosomatid parasites. SHERLOCK combines pre-amplification of nucleic acids followed by specific recognition of the desired RNA regions by the CRISPR-Cas13a system. Targeting the *Trypanosoma brucei* gambiense-specific glycoprotein (TgSGP) and the T. b. rhodesiense-specific serum resistance associated gene (SRA) allowed us to discriminate between the two species with a higher sensitivity compared to qPCR. We have now optimized SHERLOCK to detect most human-infective trypanosomatid species using an 18S guide by selecting conserved sequences among different trypanosomatid species - Pan-Trypanozoon or Pan-trypanomatid (including at least Trypanozoon subgenus, *Leishmania* sspp. and *T. cruzi*), or non-conserved 18S sequences for the detection of T. congolenese or T. vivax. Furthermore, to achieve a point-of-care test, we are optimizing the SHERLOCK technology by adapting the pre-amplification step from RPA to LAMP, as well as modifying the reaction conditions to achieve a more cost-effective technique with less equipment and pipetting, and less time consumed.

7 TT2H Fexinidazole survival genes implicate novel redox pathway in drug resistance

Arthur Drouaud (George Washington University); Jenna Murrow (George Washington University); Galadriel Hovel-Miner (George Washington University)

Fexinidazole has emerged as the front-line treatment against African trypanosomiasis, offering the first oral monotherapy against *T. brucei* gambiense infections. Because of its current success in African trypanosome treatment, fexinidazole is actively being investigated as a therapy against American trypanosomiasis (Chagas) and Leishmaniasis, which currently infect over 15 million people globally. Fexinidazole joined nifurtimox (NECT combination therapy, HAT) and benznidazole (Chagas disease) as clinically significant nitroaromatic compounds for the treatment of trypanosomatid infections. Nitroheterocyclic compounds function as prodrugs that require nitroreductase (NTR) enzymatic activation to generate cytotoxic species that are thought to damage DNA, lipids, and proteins. Naturally occurring and laboratory resistance and cross-resistance have been documented based on *T. brucei* NTR mutants and in RNAi-based knock-down studies, respectively. Fexinidazole is a repurposed compound whose success in clinical trials enabled rapid approvals with few publications evaluating the drugs trypanocidal phenotypes, Mechanism of Action (MoA), or potential for drug resistance. Inadequate molecular understanding of fexinidazole's MoA and modes of drug resistance represent a significant knowledge gap. To fill this gap, our state-of-the-art *T. brucei* Gain-of-Function library was applied to the question of fexinidazole resistance and preliminary studies identified 11 survival associated ORFs including oxidoreductases, a peroxidase, dithiol glutaredoxin, and a glutathione S-transferase. Collectively, the identified genes support the hypothesis that novel fexinidazole survival genes constitute a redox-based pathway able to promote nitroaromatic drug resistance with implications for fexinidazole's MoA.

8 TT2I A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease

Tamara Sternlieb (Institute of Parasitology, McGill University); Rhiannon Heslop (Institute of Parasitology, McGill University); Mira Loock (Institute of Parasitology, McGill University); Luisa Berenguer Antunes (Institute of Parasitology, McGill University); Igor Cestari (Institute of Parasitology, McGill University;Division of Experimental Medicine, McGill University)

Chagas disease (ChD) affects about 8 million people and is caused by infection with the protozoan parasite Trypanosoma cruzi. The infection results in an acute disease with flu-like symptoms that often develop into a chronic and lethal cardiac disease. There are no vaccines for ChD. The available drugs, benznidazole or nifurtimox, are highly toxic and their efficacy is limited. To identify potential vaccine targets for ChD, we generated a *T. cruzi* genome-wide library for yeast surface display (YSD) for screening parasite proteins that react with ChD patient's antibodies. The genome-wide library fragments range from 0.5 to 3 kb and represents 24-fold of the parasite genome. Nanopore sequencing confirmed that the library covered all chromosomes, and each parasite gene was represented by multiple clones. Computational prediction of the library-expressed proteins showed over 83,000 unique polypeptides, of which some might help identify immunogenic epitopes. Using western blot, flow cytometry, and imaging analysis we confirmed the surface expression of library proteins in yeast. Analysis of the *T. cruzi* YSD library against chronic ChD patients' sera showed that 5-10% of the yeast reacted with patients' antibodies, whereas about 1% reacted with sera of healthy subjects. Three rounds of YSD enrichment with patients' sera using magnetic-assisted cell sorting resulted in ~7-fold enrichment of the library with ~40% of the YSD population reacting with ChD patient antibodies. Using nanopore sequencing, we are identifying the *T. cruzi* antigens targeted by chronic ChD patient antibodies. We will validate the identified antigens as potential vaccine targets in animal models.

9 TT2J Structure-guided discovery of a small molecule inhibitor targeting *Trypanosoma brucei* Replication Protein A1

Aditi Mukherjee (Rutgers University, PHRI); Zakir Hossain (Queens College); Junyong Choi (CUNY Graduate Center, Queens College); Hee-Sook Kim (Rutgers University, PHRI)

DNA replication proteins are attractive targets for cancer therapies and antimicrobials because of their essential functions in cellular proliferation and viability. Replication protein A (RPA), consisting of RPA1, 2 and 3 subunits, is a eukaryotic ssDNA-binding protein and is essential for DNA replication and repair. *T. brucei* also contains all three subunits of RPA complex. Given that TbRPA1 has only 36% sequence similarity to human RPA1, we reasoned the feasibility of identification of TbRPA1-specific inhibitors. From the structure modeling and induced-fit docking analysis, we discovered a small molecule inhibitor of TbRPA1 (JC-229). The inhibitor targets the ssDNA-binding activity of TbRPA1, exhibiting 25 nM and 240 nM IC50 on purified TbRPA complex and TbRPA1 DNA-Binding-Domain (DBD) A&B (TbRPA1-DBD-AB), respectively. Interestingly, JC-229 showed no inhibition to the purified HsRPA complex or HsRPA1-DBD-AB even at 100 μM. Surprisingly, JC-229 showed no inhibitory effect on purified *T. cruzi* or L. mexicana DBD-AB proteins, although they have ~80% sequence identity. From the molecular modeling of JC-229 with Tb, Tc, and LmexRPA1-DBD-AB, we found that TbRPA1-Ser105 residue is responsible for this discrepancy and biochemically confirmed that the domain A of TbRPA1 contains a JC-229-binding pocket. JC-229-treated *T. brucei* cells behaved similarly as TbRPA1-depleted cells, exhibiting a strong inhibition of DNA synthesis. Lastly, JC-229 exhibited 6.6 μM EC50 on *T. brucei* growth, while showing ~ 20 μM and ~ 40 μM EC50 on HEK293 and HeLa cells, respectively. Here, we will discuss about JC-229's potential as a therapeutic tool for the trypanosomiasis treatment in the future.

10 TT3F Investigating trans-regulators of the *Leishmania* lifecycle

Ewan Parry (University of York); Natalia M.M. Teles (University of York); Rachel Neish (University of York); Katherine Newling (University of York); Michael J. Plevin (University of York); Angela K. Cruz (University of São Paulo, Ribeirao Preto); Jeremy C. Mottram (University of York); Pegine B. Walrad (University of York)

Leishmaniasis cases are rising globally, no vaccine is currently available and limited treatments have toxic side effects and growing resistances. *Leishmania* parasites transition between extracellular stages in the sandfly to an intracellular stage that infects and destroys human immune cells. Fundamental parasite processes can inform new intervention strategies. Lifecycle progression requires tightly-coordinated gene expression that preadapts *Leishmania* parasites for survival and virulence. Kinetoplastid gene regulation is predominantly reliant on post-transcriptional mechanisms. Accordingly, RNA binding proteins (RBPs) have an elevated importance for gene regulation in these organisms. Despite this, strikingly few trans-regulators have been characterised in *Leishmania* to date. Recently we isolated and compared the mRBPome of the 3 main *Leishmania* lifecycle stages and found that RBPs selectively bind distinct transcriptomes in a stage-specific manner. Moreover, a separate study revealed that arginine methylation of RBPs impacts target transcript association and stability in a method both bespoke and precise with epigenetic implications toward parasite virulence. Building upon this work, a bar-coded trans-regulator knockout clone library was created and screened for essential roles in lifecycle progression and macrophage/mouse infections. Of those screened, 40 RBPs were incapable of creating viable knockout cell lines and 18 contribute to cell differentiation to human-infective stages and/or parasite infectivity. Examination of individual knockout lines for amastigote-specific RBPs showed normal promastigote growth dynamics, whereas infection in peritoneal macrophages was inhibited. This suggests essential roles for these RBPs in amastigote viability and virulence. Experiments are underway to identify the novel virulence pathways these RBPs regulate. Our findings link *Leishmania* trans-regulatory networks to eukaryotic post-transcriptional epigenetics.

11 TT3G Genome-scale RNA interference profiling of *Trypanosoma brucei* cell cycle progression defects

Melanie Ridgway (University of Glasgow); Catarina A Marques (University of Glasgow); Michele Tinti (University of Dundee); Andrew Cassidy (University of Dundee); David Horn (University of Dundee)

Trypanosomatids, which include major pathogens of humans and livestock, are divergent eukaryotes for which cell cycle controls and the underlying mechanisms are not completely understood. Here, we describe a genome-wide RNA-interference library screen for cell cycle defects in *Trypanosoma brucei*. We induced massive parallel knockdown, sorted the perturbed population using high-throughput flow cytometry, deep-sequenced RNAi-targets from each stage and digitally reconstructed cell cycle profiles at a genomic scale; also enabling data visualisation using an online tool (https://tryp-cycle.pages.dev/). Analysis of several hundred genes that impact cell cycle progression revealed over 100 flagellar component knockdowns linked to genome endoreduplication, evidence for metabolic control of the G1-S transition, surface antigen regulatory mRNA-binding proteins required for cytokinesis, and a putative nucleoredoxin required for both mitochondrial genome segregation and for mitosis. The outputs provide comprehensive functional genomic evidence for the known and novel machineries, pathways and regulators that coordinate trypanosome cell cycle progression.

12 TT3H Thousands of messenger RNA untranslated regions reprogram post-transcriptional gene expression profiles in trypanosomes

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

Cells express thousands of proteins that differ in abundance over a wide range. In nucleated cells, messenger RNA untranslated regions (UTRs) can contribute to post-transcriptional expression control, but relatively few UTRs have been shown to exert these controls, and cis-regulatory sequences have remained largely unknown. In the trypanosomatids, several of which are important human and animal parasites, genome-wide polycistronic transcription places a particular emphasis on post-transcriptional controls. Here, we describe a massive parallel reporter assay coupled with UTR-seq profiling in the African trypanosome, revealing post-transcriptional reprogramming by thousands of 3'-UTRs. Genome-scale UTR-seq screening identified an abundance of regulatory fragments that either increased or reduced reporter expression. Analysis of regulatory fragments and native UTRs yielded a correlation between gene expression, 3'-UTR length and adenine (A)-rich poly-purine tract abundance, as well as a more widespread enrichment of thymine (T)-rich poly-pyrimidine tracts in UTRs. A machine learning approach guided by these findings effectively predicted observed measures of translation efficiency at a transcriptomic scale; R2 was 0.69. This approach also provided quantitative measures of the relative contribution of sequences within native 3'-UTR and revealed similarly predictive sequences within 5'-UTRs. Thus, we describe the cis-regulatory UTR sequences that control gene expression in the context of a genome that lacks promoter-based transcription control. We conclude that gene expression profiles in trypanosomes are post-transcriptionally reprogramed by thousands of poly-purine tract biased UTRs.

13 TT3I The role of KPAP1 methylation in transcript-specific mitochondrial mRNA tail addition in *Trypanosoma brucei*

Clara Smoniewski (University of Minnesota Medical School, Duluth campus); Poorya Mirzavand Borujeni (McGill University); Marshall Hampton (University of Minnesota Duluth); Reza Salavati (McGill University); Sara L Zimmer (University of Minnesota Medical School, Duluth campus)

Trypanosoma brucei mitochondrial mRNAs have non-templated heterogenous adenine/uridine tails added post-transcriptionally to their 3' ends. We previously found population level differences in tail characteristics, such as length and composition, between transcripts. The mechanism(s) for these differences is not established. Tail adenines are added by kinetoplast poly(A) polymerase I (KPAP1), possessing two previously identified arginine methylation sites. Methylation of protein arginine residues is a known mechanism for altering protein:protein and protein:nucleic acid associations. We have mutated KPAP1 at its arginine methylation sites to mimic constant methylation (methyl-mimic) or no methylation (methyl-mutant). These mutated versions of KPAP1 and a wildtype version were inducibly expressed in procyclic *T. brucei* along with simultaneous silencing of native KPAP1. The three distinct cell lines were then compared. Using circTAIL-seq, which combines enzymatic circularization of RNA, RT-PCR and Illumina sequencing, we found that some transcripts' tail characteristics are affected by methyl-mimic or methyl-mutant KPAP1. This suggests a role for methylation in KPAP1 regulation. Surprisingly, some transcripts yielded slightly shorter circTAIL-seq libraries when the ligase responsible for RNA circularization was omitted as a negative control. Sequencing reveals that these libraries consist of cDNAs possessing 3' and 5' UTRs of the transcript of interest, often truncated, sometimes possessing a short string of adenines and/or uridines between the two UTRs. We are currently pursuing additional approaches to determine whether these products reflect a minor sub-population of the mitochondrial mRNAs that are intracellularly circularized. To our knowledge, this would be the first circular RNA to be identified in a Kinetoplastid species.

14 TT3J Biogenesis of trypanosomal mitoribosomes employs conserved and lineage-specific mechanisms and multitude of assembly factors

Ondřej Gahura (Biology Centre of the Czech Academy of Sciences); Jonathan Wong (Biology Centre of the Czech Academy of Sciences); Prashant Chauhan (Biology Centre of the Czech Academy of Sciences); Shintaro Aibara (Science for Life Laboratory, Stockholm University); Vivek Singh (Science for Life Laboratory, Stockholm University); Alena Zíková (Biology Centre of the Czech Academy of Sciences); Alexey Amunts (Science for Life Laboratory, Stockholm University)

Mitoribosomes in trypanosomatid parasites diverged markedly from their bacterial ancestors and other eukaryotic lineages in terms of protein composition, rRNA content and overall architecture. We and others obtained cryoEM structures of several native precursors of large and small mitoribosomal subunits (mtLSU and mtSSU), which revealed that biogenesis of trypanosomatid mitoribosomes employ intricate machineries containing a multitude of assembly factors. We showed that the assembly of *T. brucei* mtSSU requires a homolog of RbfA, acting in biogenesis of bacterial ribosomes and mammalian mitoribosomes. Conserved core of TbRbfA binds several key rRNA helices of the decoding center and mRNA channel and together with long lineage-specific extensions at both termini stabilizes the immature interface of all four domains of mtSSU rRNA. The C-terminal extension also serves as a binding platform for a heterodimer of two conserved methyltransferases and a kinetoplastid-specific pentameric assembly factor mt-SAF24. The N-terminal domain of mt-SAF24 forms a protrusion on the prospective intersubunit interface, which is capped by a disc-shaped density of unknown composition. By combining in vivo and in vitro approaches, we test the hypothesis that the density represents a piece of phospholipid bilayer and that the biogenesis of mtSSU occurs in the association with the inner mitochondrial membrane. Comparison of the trypanosomal pre-mtSSU complexes with a recently reported series of mammalian mtSSU precursors revealed common assembly mechanisms and allowed us to infer a chimeric pathway for mtSSU assembly and propose that the exit part of the mRNA channel in trypanosomal mitoribosome is deviated compared to other ribosomes.

15 Amino acid uptake in *Leishmania* virulence

Jamal Asseri (University of Glasgow); Richard Burchmore (University of Glasgow); Harry De-koning (University of Glasgow)

Leishmaniasis is a potentially fatal disease caused by insect vector-borne parasite *Leishmania* and found in over 80 countries across the world. Currently available treatments for Leishmaniasis are insufficient to meet the needs of the growing public health problem caused by the disease, and the development of novel, potentially more effective therapeutics is a key area of research in parasitology. Like many other parasites, *Leishmania* spp. have lost the ability to synthesise some nutrients de novo and need to scavenge certain amino acids from their external environment. Salvage of these essential amino acids occurs through various pathways involving amino acid transporter (AAT) proteins found on the membranes of the parasite that interact with the external environment. The routes by which amino acids are salvaged and metabolised represent potential therapeutic targets. However, the majority of AATs involved in amino acid salvage by *Leishmania* spp. have yet to be fully characterised. This project aims to build upon the research completed so far by identifying the functional roles of putative AATs used by *L. mexicana* promastigotes.

16 Cracking the vault

Anna Zavřelová (Faculty of Science, Charles University in Prague); Siqi Shen (Faculty of Science, Charles University in Prague); Martin Zoltner (Faculty of Science, Charles University in Prague)

Many, but not all Eukaryotes have protein-enclosed compartments called vaults. Vaults are composed of multiple copies of the major vault protein (MVP), symmetrically assembled into a basket-like shell. A human cell contains approximately 100,000 vaults, localized mainly to the cytosol but also to the nucleus and nuclear pore. Whilst there is some evidence that these compartments are engaging in mRNA maturation and intriguing structural information of the vault shell is available, the function of vaults is largely elusive. We set out to address this fundamental knowledge gap by targeting the vault of *Trypanosoma brucei* by interactomics studies. First, we performed endogenous GFP-tagging of one of the three MVP paralogs (Tb927.5.4460) present in *T. brucei*. We obtained GFP-tagged vault particles upon N-terminal fusion as monitored by immunofluorescence microscopy. Next, we performed cryomill affinity enrichment proteomics experiments using magnetic anti-GFP nanobody beads under three extraction conditions with different stringency. Significantly, we detected the other two MVP paralogs strongly suggesting that vault particles are composed of all three paralogs of the MVP. Apart from this finding the different extraction condition returned different potential interactors: under stringent conditions we detected fewer candidate interactors, including telomerase-associated protein 1 (TEP1), which is a known vault component. In less stringent conditions we found many proteins including helicases and DNA repair proteins. These could be transient interactors of the MVP. Currently we are trying to confirm the interactome by engineered ascorbate peroxidase (APEX)-based proximity labelling using MVP and potential interactors identified by affinity capture as bait.

17 Probing the role of regulation loss in compartmentalized phosphofructokinase in trypanosomatids

Austin Petersen (University of Minnesota Duluth); Jon Rumbley (University of Minnesota College of Pharmacy, Duluth Campus); Sara L Zimmer (University of Minnesota Medical School, Duluth Campus)

Trypanosoma brucei is a kinetoplastid and thus possesses an organelle called a glycosome that is common to this group. The glycosome is specialized, in part, to compartmentalize the first steps of glycolysis, rather than having the full glycolytic pathway exist in the cytosol as in other eukaryotes. Compartmentalized *T. brucei* phosphofructokinase (TbPFK), the third enzyme of the glycolytic pathway, has lost sensitivity to allosteric effector molecules normally necessary to control glycolysis when it occurs entirely in one compartment. Thus, its activity is largely unregulated. We seek to better understand the cellular fitness impacts of compartmentalizing PFK, including this loss of sensitivity. This is particularly important as recent evidence suggests that the compartmentalized PFKs of other disease-causing kinetoplastids do not exhibit the same degree of insensitivity to allosteric effectors as TbPFK. It is possible that TbPFK loss of regulatability is due to genetic drift, as compartmentalization removed the selective pressure to maintain regulatability. However, we hypothesize that the degree to which PFK is sensitive to allosteric effector molecules phosphoenolpyruvate and AMP in various kinetoplastids confers a fitness advantage. We are taking an in vivo approach to test this hypothesis. Upon silencing of native TbPFK, we concurrently express TbPFK (add-back), regulatable Escherichia coli PFK, and semi-sensitive *Leishmania mexicana* PFK. Growth analysis of the cell lines suggests that restoring TbPFK with more regulatable versions negatively impacts organism fitness. We will next test the various strains for the rate at which they can generate ATP, and other metabolic considerations.

18 Expanding the unusual mRNA export platform of Trypanosomes

Bernardo Papini Gabiatti (University of Würzburg); Silke Braune (University of Würzburg); Timothy Krüger (University of Würzburg); Johanna Odenwald (University of Würzburg); Susanne Kramer (University of Würzburg)

All trypanosomatid mRNAs are processed from large polycistronic transcription units by trans-splicing. Inhibition of trans-splicing increases unprocessed transcripts and induces RNA-protein granules at the nuclear periphery (NPGs). The function of NPGs remains unknown but their position, composition and dependency on unprocessed mRNAs suggests involvement on nuclear export control. Trypanosomes lack several protein complexes and nuclear pore associated proteins with major roles in mRNA export in Opisthokonts. The parasites can even start nuclear export before transcription is finished. Still, putative analogues to proteins of the nuclear basket, which in yeast dynamically interacts with pores and retains unprocessed transcripts, are present (NUP110). To understand the relationship between NPGs and mRNA export control, we visualized these components by expansion microscopy. We achieved 3.5-fold isotropic expansion, were able to resolve and quantify individual pores and, for the first time, pore-density on the trypanosome nucleus. Moreover, we resolved distances between NUP110, the core nuclear pore and NPGs. We confirmed previous findings with electronic microscopy that the NUP110 is at the nuclear face of the pore only. However, in contrast to the yeast basket, localisation is not affected by a block in mRNA transcription or processing and the protein is present at all nuclear pores, including the ones close to the nucleolus. Our data show that the trypanosome basket-like structure may be functionally different and not as dynamic as the Opisthokont counterpart. We will now use the system to investigate phenotypes of cells depleted in proteins with known or suspected functions in mRNA export. Our data will contribute to a better understanding of nuclear pore organization and nuclear export evolution.

19 The molecular basis of heat shock signalling in African trypanosomes

Caroline Dewar (Lancaster University); Marianne Aelmans (Lancaster University); Mick Urbaniak (Lancaster University)

The heat shock (HS) response represents a significant virulence factor for African trypanosomes, with disease manifesting in the mammalian host as periodic fever which is sensed by the parasites, requiring adaptation to this stress. Eukaryotic cells typically trigger general protein translation arrest to prevent accumulation of misfolded proteins, whilst increasing the expression of proteins which aid protein folding and degradation. While a HS response does occur in *T. brucei*, the molecular mechanisms mediating this response are not understood. Transcription factors are not present to mediate a global response to the stimulus, and translational arrest is independent of the mechanism found in mammals. Preliminary evidence suggests regulation occurs through a novel post-transcriptional mechanism involving dynamic phosphorylation of unique regulatory RNA binding complexes. We aim to combine global quantitative proteomics with phosphoproteomics to kinetically profile trypanosome HS signalling, and examine the role of specific phosphorylation events on post-transcriptional regulators. We are examining the HS response in both *T. brucei* and the less well characterised *T. congolense*, the leading cause of AAT (Animal African Trypanosomiasis), utilising novel genetic manipulation techniques now possible in this organism. These parasites co-infect the same hosts with exposure to similar evolutionary selective pressures, and it is expected they will show some similarities in their host interactions. We will show data comparing the HS response of both parasites in terms of broad characteristics, and the relocalisation and/or upregulation of key components. We also examine the influence of key early phosphorylation sites in progression through the HS response.

20 Genetic deletion of pyrroline-5-carboxilate synthetase and pyrroline-5-carboxylate reductase suggests a shortcut in the glutamate-proline pathway and the direct use of glutamate as energy source in *Leishmania braziliensis*

Gustavo Daniel Campagnaro (Ribeirão Preto Medical School - USP); Angela Kaysel Cruz (Ribeirão Preto Medical School - USP)

Proline (Pro) catabolism provides substrates for the tricarboxylic acid cycle. *Trypanosoma brucei* is auxotrophic for Pro, whereas *T. cruzi* is capable of both Pro uptake and de novo biosynthesis from glutamate (Glu) via the activity of pyrroline-5-carboxilate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), which enables it to use Glu as sole Carbon source. Starved (4h in PBS) procyclic promastigotes (PRO) of *Leishmania braziliensis*, L. amazonensis and *L. major* recovered mitochondrial function with various Carbon sources, including Glu, suggesting a functional de novo Pro biosynthesis pathway. In L. *braziliensis*, mRNAs encoding for LbrP5CS and LbrP5CR are found in PRO, metacyclic (META) and axenic amastigote (AMA) forms, being elevated in META. myc-tagged LbrP5CS and LbrP5CR, however, showed a variable pattern of expression across different biological forms, with LbrP5CS expression reduced in META and absent in AMA, whereas LbrP5CR expression was detected in all three life cycle stages with highest levels in META. Surprisingly, the knockouts (KOs) of LbrP5CS and LbrP5CR did not alter parasite ability to efficiently use Glu as energy source. Parasites KO for Pro dehydrogenase, the first catabolic enzyme of Pro, could utilize Glu, but not Pro, as the sole energy source. Furthermore, starved L. *braziliensis* recovered in Glu did not restore their intracellular free Pro levels. Altogether, our results indicate that the Pro biosynthesis is unlikely to be functional in L. *braziliensis* and, instead, energy generation from Glu follows a distinct route from the Pro pathway.

Poster Session B Abstracts

Thursday 7 PM
September 15th 2022

21 Synthetic glycovaccines elicit cross-protection against Chagas disease and cutaneous Leishmaniasis in a murine co-infection model.

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Chagas disease (CD) and cutaneous Leishmaniasis (CL) are two neglected tropical diseases caused by the protozoan trypanosomatids, *Trypanosoma cruzi* and *Leishmania spp.*, respectively. Currently, ~7 million people are infected with CD worldwide, with ~300,000 chronically infected patients in the US. Between 700,000 and 1.2 million new cases of CL occur worldwide yearly. The approved chemotherapies for both diseases may cause serious adverse events, resulting in premature treatment interruption. Moreover, no effective vaccine for either disease is available. Therefore, developing a vaccine that would provide effective cross-protection against both diseases would provide a cost-effective alternative to the toxic chemotherapies currently in use. Due to the evolutionary loss of the a1,3-galactosyltransferase (a1,3GT) gene in humans and Old-World nonhuman primates, α-Gal epitopes are highly expressed on both *Trypanosoma cruzi* and *Leishmania* spp. cell surfaces are highly immunogenic. We have previously shown that synthetic a-Gal-based vaccines can effectively protect against either murine acute *T. cruzi* or *L. major* infection. We, therefore, hypothesize that a synthetic glycan-based vaccine could provide effective cross-protection against a *T. cruzi* and *L. major* co-infection. Here we use an a1,3GT-knockout (a1,3GT-KO) murine model to evaluate the efficacy of two new synthetic neoglycoproteins, NGP28b, and NGP29b, containing nonreducing, terminal a-Gal and b-Galf glycotopes, respectively, found in both parasites and absent in the a1,3GT-KO mouse, as potential vaccine candidates against CD and CL. Our data indicate that both vaccine candidates induce a strong cross-protective immune response, significantly reducing parasite burden and increasing animal survival in this co-infection experimental model.

22 Cytokinesis in *Trypanosoma brucei* relies on an orphan kinesin that dynamically crosslinks microtubules

Thomas E Sladewski (University of Connecticut); Paul C Campbell (Brown University); Neil Billington (NHLBI); Alexandra D'Ordine (Brown University); Christopher L de Graffenried (Brown University)

Many single-celled eukaryotes have complex cell morphologies defined by microtubules arranged into higher-order structures. *Trypanosoma brucei* (*T. brucei*) cell polarity is mediated by a parallel array of microtubules that underlie the plasma membrane and define the auger-like shape of the parasite. The subpellicular array must be partitioned and segregated using a microtubule-based mechanism during cell division. We previously identified an orphan kinesin, KLIF, that localizes to the ingressing cleavage furrow and is essential for the completion of cytokinesis. We have characterized the biophysical properties of a truncated KLIF construct in vitro to gain mechanistic insight into the function of this novel kinesin. We find that KLIF is a non-processive dimeric kinesin that dynamically crosslinks microtubules. Microtubules crosslinked by KLIF in an antiparallel orientation are translocated relative to one another, while microtubules crosslinked parallel to one another remain static, resulting in the formation of organized parallel bundles. In addition, we find that KLIF stabilizes the alignment of microtubule plus ends. These features provide a mechanistic understanding for how KLIF functions to form a new pole of aligned microtubule plus ends that defines the shape of the new cell posterior, which is an essential requirement for the completion of cytokinesis in *T. brucei*.

23 On the trail of the new jobs of TbPam18 and TbPam16

Corinne von Känel (Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern); Silke Oeljeklaus (Biochemistry and Functional Proteomics, Institute of Biology II, Faculty of Biology and Signalling Research Centres BIOSS and CIBSS, University of Freiburg); Salvatore Calderaro (Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern); Bettina Warscheid (Biochemistry and Functional Proteomics, Institute of Biology II, Faculty of Biology and Signalling Research Centres BIOSS and CIBSS, University of Freiburg); André Schneider (Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern)

Up to 99% of all mitochondrial proteins are encoded in the nuclear genome, synthesized in the cytosol, and then imported into mitochondria. Mitochondrial protein import has best been studied in the yeast Saccharomyces cerevisiae and much of this work can be generalized to most eukaryotic lineages. However, analysis of the protein import machinery in the parasitic protozoan *Trypanosoma brucei* revealed that its import complexes are highly diverged from the ones found in other eukaryotes. In yeast, the driving force to import proteins into the mitochondrial matrix is mainly provided by a complex termed presequence translocase-associated motor (PAM). Several homologues of PAM components can be identified in *T. brucei*. Among these are orthologues of the yeast PAM subunits Pam18 and Pam16, termed TbPam18 and TbPam16. Surprisingly, TbPam18 and TbPam16 are not associated with the trypanosomal PAM and not involved in mitochondrial protein import. Instead, we show that TbPam18 and TbPam16 play lifecycle-specific roles in the replication of the mitochondrial DNA. The single-unit mitochondrial genome of *T. brucei*, termed kinetoplast DNA, consists of a complex network of two types of interlocked DNA rings, the mini- and maxicircles. Intriguingly, TbPam18 and TbPam16 seem to be specifically involved in maxicircle replication.

24 Single cell transcriptomic analysis of the *T. brucei* cell cycle and differentiation deciphers cell cycle progression and exit

Emma Marie Briggs (University of Edinburgh); Catarina Marques (University of Glasgow); Thomas Dan Otto (University of Glasgow); Keith R Matthews (University of Edinburgh)

The African trypanosome life cycle involves differentiation transitions between replicative and cell cycle arrested forms, the latter of which are often primed for transmission between the mammal and tsetse fly. Single cell transcriptomics allows the dissection of mixed populations, both in terms of life cycle and cell cycle stages, bioinformatically and without prior cell sorting, and has been applied successful to several stages of the *T. brucei* life cycle. We have applied Chromium scRNA-seq to in vitro replicating monomorphic bloodstream form and procyclic form *T. brucei* to profile cell cycle transcript changes in new detail. Using non-linear trajectory inference and pseudotime analysis we find around ~1,000 genes regulated at the transcript level in both or either life cycle form. We additionally resolve the dynamic transcript level patterns over the cell cycle to classify genes based on expression during distinct phases, and during the transitions between phases. To explore the temporal relationship between transcript and protein levels, we compare transcript dynamics during the cell cycle with previously reported protein abundances (Crozier et al Mol Cell Proteomics, 2018). We find that most genes regulated at both the transcript and protein levels display a temporal delay, yet the extent of delay is variable between genes, highlighting the multiple levels of gene expression control in *T. brucei*. Comparison of transcript level regulation between the monomorphic and pleomorphic bloodstream form cell cycles, highlights key putative cell cycle regulators of G1 exit in pleomorphs. Experimental validation of these potential check point regulators is ongoing.

25 The histone methyltransferase DOT1B is dispensable for stage differentiation in *Leishmania mexicana* but not for efficient infection of macrophages

Elisa Theres Rauh (Department of Cell & Developmental Biology, Biocenter, University of Würzburg); Nicole Eisenhuth (Department of Cell & Developmental Biology, Biocenter, University of Würzburg); Falk Butter (Quantitative Proteomics, Institute of Molecular Biology (IMB), Mainz); Andrea Debus (Microbiology Institute-Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander-University, Erlangen); Ulrike Schleicher (Microbiology Institute-Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander-University, Erlangen); Christian J. Janzen (Department of Cell & Developmental Biology, Biocenter, University of Würzburg)

Post-translational histone modifications such as methylation of lysine or arginine residues are used to regulate many nuclear processes such as transcription, DNA repair and replication in eukaryotes. DOT1 (disruptor of telomeric silencing 1) is a conserved histone methyltransferase that mediates methylation of lysine 79 of histone H3 (H3K79me) in many eukaryotes. In trypanosomatids, two homologues of DOT1, DOT1A and DOT1B, have been identified and are best characterised in *Trypanosoma brucei*. While TbDOT1A mediates mono- and di-methylation of H3K76, TbDOT1B can additionally catalyse tri-methylation of this residue (H3K76me3). TbDOT1A is necessary for replication initiation and thus essential for the survival of the cells. TbDOT1B is not essential for parasite growth but is required for efficient in situ switching of variant surface glycoproteins. Interestingly, TbDOT1B-depleted bloodstream forms are not able to differentiate to procyclic forms. Here, we aimed to elucidate whether the functions of DOT1 histone methyltransferases are conserved in *Leishmania mexicana*. As in *T. brucei*, the H3K76 methylation pattern is also cell cycle-regulated in *L. mexicana*. LmxDOT1B is not essential for growth of procyclic promastigotes in vitro. LmxDOT1B deletion resulted in a loss of H3K76me3, indicating that LmxDOT1B is solely responsible for this histone modification. Strikingly, and in contrast to *T. brucei*, LmxDOT1B is not essential for differentiation of axenic procyclic promastigotes to amastigotes in vitro, but important to establish an infection of bone marrow-derived macrophages. These data suggest that although the enzymatic activity of LmxDOT1 enzymes is conserved*, L. mexicana* parasites have developed novel functions specific to their life cycle.

26 Exploring the cellular targets of anti-Leishmanial natural product analogues using fluorescence microscopy

Hannah A Asiki (University of Oxford); Richard J Wheeler (University of Oxford); Edward A Anderson (University of Oxford)

The dehydrodieugenol family of natural products have specific activity against *L. donvani*, *L. infantum* and L. mexicana. This prompted synthesis of related analogues, with improved activity now comparable to Miltefosine. Initial evidence suggests these compounds affect the mitochondrion, however evidence is weak and the protein target(s) are unknown. As the first of several approaches for insight into targets we evaluated fluorescent compound derivatives, using 3 approaches: 1- Direct attachment of fluorescein/rhodamine, 2- Attaching an alkyne “handle” used for an in situ click reaction to attach a fluorescent azide and 3- An alkyne and diazirine handle allowing in situ photocrosslinking to proteins and click. We assessed compound activity and determined compound subcellular localisation in *L. mexicana* promastigotes. Direct attachment of fluorescein/rhodamine lead to compound inactivity, while photocrosslinking was necessary to prevent compound wash-out during the click protocol and allow images to be captured. Fluorescence appeared mitochondrial, which using the power of CRISPR/Cas9 genetic modifications we are confirming with mNeonGreen co-localisation. Pulse-chase experiments with the photocrosslinking derivative show stable accumulation in live cells compared to fixed cells indicating an active process. These derivatives can be used for in situ biotin click as a toolkit for affinity purification and related approaches. This technique combined with parallel approaches including selecting for drug resistant mutants will allow us to evaluate protein targets of this family of compounds.

27 TT4G A Global Perspective on the Evolutionary Impetus for Glycosomes in Kinetoplastea

Hina Durrani (University of Minnesota); Sara L Zimmer (University of Minnesota)

The first steps of glycolysis are compartmentalized in kinetoplastids, to an organelle called the glycosome. A predominant hypothesis regarding the evolutionary benefit of glycosomes is that it facilitates efficient adaptation of the glycosomal metabolic repertoire. Turnover and replacement of glycosomes with those possessing a different enzyme composition is thought critical for sudden, major nutritional changes encountered throughout a kinetoplastid life cycle. This hypothesis is based mainly on *Trypanosoma brucei*, which can generate energy through cytochrome-mediated respiration in its Tsetse fly host but when replicating in a mammalian bloodstream generates energy only through glycolysis. Our objective is to establish the level of empirical support for this hypothesis across the Kinetoplastea. One approach is to determine the extent to which culturable kinetoplastids of various lifestyles execute a change their glycosomal enzyme composition in response to extracellular signals. We are initially pursuing glucose concentration as an extracellular signal, by modulating culture glucose for six kinetoplastids. Our selected species grow at different rates and to different maximal abundances, but all exhibited attenuated growth in low or no glucose. We measure abundances of likely-glycosomal enzymes in these species growing in consistent regular, low, and no glucose culture conditions, and during transition in concentration. When glucose is absent, at least some glycolysis enzymes appear less abundant in *T. brucei*, *Trypanosoma cruzi* and *Leptomonas pyrrhocoris*, but enzyme abundances of other species such as *Leptomonas barvae* appear largely insensitive to extracellular glucose concentration. Likewise, species range in sensitivity of enzyme abundance response to changes in glucose. Initial results suggest we revisit the origins and benefits of kinetoplastid glycosome enzyme compartmentalization.

28 KRGG1 is essential and required for normal editing in bloodstream form *Trypanosoma brucei*

Jason Carnes (Seattle Children's Research Institute); Claire Gendrin (Seattle Children's Research Institute); Suzanne M. McDermott (Seattle Children's Research Institute); Ken Stuart (Seattle Children's Research Institute)

Mitochondrial gene expression in trypanosomes requires numerous multiprotein complexes that are unique to kinetoplastids. Among these, the most well characterized are RNA Editing Catalytic Complexes (RECCs) that catalyze guide RNA (gRNA)-specified insertion and deletion of uridines during mitochondrial mRNA maturation. This post-transcriptional resequencing of mitochondrial mRNAs can be extensive, involving dozens of different gRNAs and hundreds of editing sites, with most of the mature mRNA sequence resulting from the editing process. Proper coordination of gRNA recognition of their cognate mRNA sequences is attributed to RNA Editing Substrate-binding Complexes (RESCs), which are also required for RNA editing. Although the precise mechanism of RESC function is less well understood, their affinity for binding both editing substrates and products suggests that these complexes provide a scaffold for mRNA/gRNA substrates that are available to RECCs for catalytic processing. KRGG1 has been shown to bind RNAs and its affinity purification also co-isolates RESC complexes but its role in RNA editing remains unclear. We show here that KRGG1 is essential in BF parasites and required for normal editing. KRGG1 repression results in a specific reduction of edited A6 and an increase of edited ND8 mRNAs. Sequence and structure analysis of KRGG1 reveals homology with RESC6, and both proteins have predicted tandem helical repeats similar to mTERFs. The mTERF-like region is critical for KRGG1 function, as site-directed mutants prevent growth and association with RESCs when exclusively expressed. Our results clarify the role that KRGG1 plays in RNA editing and indicate a functional mechanism related to RESC activity.

29 Does *Leishmania* ATM play a role in DNA repair and genome plasticity?

Jennifer A Black (University of Sao Paulo); Gabriel L A Silva (University of Glasgow); Luiz R O Tosi (University of Sao Paulo)

Faithful genome transmission requires the resolution of DNA injuries before cell division. Double Strand Breaks (DSBs) are highly deleterious lesions which can lead to chromosome breakage, genome instability, tumorigenesis, and cell death if unresolved. At the forefront of the cellular response to DSBs is the atypical kinase ATM (Ataxia-telangiectasia Mutated), which is recruited to DSBs via the MRN complex (Mre11-Rad50-Nbs1). Together, these events lead to a phosphorylation cascade promoting the recruitment of repair factors and thus, resolution of the DSB. Paradoxically, DSBs can also drive genome diversity, for instance during V(D)J recombination, underpinning antibody diversity. *Leishmania* possess remarkably plastic genomes but how these parasites balance plasticity whilst retaining genome stability is unclear. Furthermore, little is known about DNA lesion signalling and resolution. The function of *Leishmania* ATM has been previously linked to the response to oxidative stress but is it unclear if ATM plays wider roles, for example, tackling other types of DNA damage, during cell cycle progression or to moderate genome plasticity. Here, using CRISPR/Cas9 we deleted ATM in *L. major* promastigotes. We found that ATM, in vitro, is non-essential however, the loss of ATM moderately affects proliferation, significantly sensitises cells to genotoxins and is required for H2A phosphorylation (yH2A), a marker of DNA damage. Additionally, our data suggests ATM may moderate a damage induced G1/S checkpoint after methyl methanesulfonate (MMS) exposure. Lastly, we show evidence for copy number variations and genomic instability in ATM's absence suggesting ATM plays a role in maintaining the stability of the *Leishmania* genome.

30 Investigating parasite diversity during congenital transmission of *Trypanosoma cruzi*

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Congenital transmission of Chagas disease is an important source of new infections worldwide, though the mechanisms behind it are poorly understood. Here we use deep amplicon sequencing of a single copy gene in the *Trypanosoma cruzi* genome to assess parasite populations in infected mothers and their children. We found an increase in parasite population diversity in children after birth compared to their mothers. This increased diversity was stable in infants who were sampled longitudinally up to 3 months after birth. In many cases, mothers' parasite populations were dominated by a single parasite clone, while children had a distribution of several clones. These differences in diversity were independent of parasite load and read depth for each sample, indicating that this is likely a biological phenomenon, not a technical artifact. This could support a model in which certain parasite clones show a tropism for placental tissue. They are transmitted to and detectable within the acutely-infected infant but cannot be detected in the circulation of the mother. Additionally, the results we observe suggest that that during congenital infection, there is no transmission bottleneck for parasites infecting and successfully colonizing the infant. We are currently exploring using Nanopore sequencing with adaptive sampling to enrich for parasite genomic content in metagenomic blood samples. Generating long whole genome reads of parasite DNA would allow us to further investigate diversity between parasite clones and aid in identifying parasite genetic determinants of transmission and potential tissue tropism.

31 Automation and optimization of nucleotide extraction method to improve detection of *Trypanosoma cruzi* satellite DNA in the blood of chronically infected patients

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Currently, one of the top priorities in Chagas disease's basic and translational research is to establish accurate diagnostic tools in chronically infected animals and patients, when the parasitemia is mostly undetectable through conventional diagnostic methods. Newest advances in molecular diagnostics made possible the development of a more sensitive method for the detection and quantification of *Trypanosoma cruzi* satellite DNA by quantitative real-time PCR (qPCR). The KingFisher Duo Prime (KFDP) is a benchtop automated instrument for the extraction of nucleic acids and proteins from cells, tissues, blood, and biofluids that uses a technique based on magnetic beads. Therefore, we propose the automation of DNA extraction using the KFDP equipment, aiming to increase the yield of extraction from biosamples and, subsequently, the sensitivity of parasite DNA detection in biosamples by qPCR. Different *T. cruzi* trypomastigote concentrations were spiked in guanidine-EDTA human blood samples, followed by spiking with exogenous internal positive control. When comparing the DNA yield using Nanodrop, KFDP yielded 56.0-79.3 ng/µL, whereas High Pure PCR Template Preparation Kit (Roche) yielded 25.2-37.9 ng/µL, i.e., a 2-fold lower yield when compared with KFDP. When analyzed using Qubit, KFDP yielded 20.8-33.6 ng/µL, whereas the Roche kit yielded 2.8-7.6 ng/µL, i.e., a 10-fold lower yield in comparison with KFDP. qPCR performed in these samples showed a ~8-fold increased satDNA detection using the KFDP extraction. These results indicate that automated nucleotide extraction could improve the detection of *T. cruzi* in blood samples from experimental animals and patients.

32 Trapped in Export: NUP-mRNA interactions in trypanosomes

Johanna Odenwald (Julius-Maximilians-Universität Würzburg); Siqi Shen (Charles University Prague); Bernardo Gabiatti (Julius-Maximilians-Universität Würzburg); Claudia Moreira (Carlos Chagas Institute Curitiba); Martin Zoltner (Charles University Prague); Susanne Kramer (Julius-Maximilians-Universität Würzburg)

Trypanosomes separated from the eukaryotic main lineage early, allowing more than one billion years to develop their own, unique biology, including a highly unusual mRNA metabolism. Whilst other Eukaryotes limit nuclear mRNA export to mature, fully processed mRNAs, export in trypanosomes can start co-transcriptionally. The study of nuclear mRNA export is challenging, because it is very fast, and interactions between mRNAs and nuclear pore proteins are transient. The co-transcriptional nuclear export in trypanosomes allows us to trap mRNAs together with attached proteins (mRNPs, messenger ribonucleoprotein particles) in export, simply by blocking transcription or splicing. Here, we employ this unique trypanosome mRNA trap to ‘freeze’ nuclear export and study the otherwise very transient interactions between mRNPs and nucleoporins during mRNA export. To this end, we are currently establishing a range of proximity labelling techniques for proteins and RNA, complemented by classical biochemical purification approaches in order to map the interactions between nuclear pore proteins, mRNPs and mRNAs in transit. Initital experiments with TurboID indicate a small labelling radius with specific labelling of sub-complexes of the nuclear pore with clear differences in the interactomes between core nuclear pore proteins and the nuclear export receptor MEX67. The combined proteomic and transcriptomic data will provide insight into the details of nuclear mRNA export and possible control mechanisms in trypanosomes. A comparison of conserved and trypanosome-unique features of the nuclear export pathway will advance our knowledge on the evolution of this pathway.

33 Overexpression of RNA Editing Helicase 2 (KREH2) Wild-Type and Dominant Negative Disrupts Editing in African Trypanosomes

Joshua Meehan (Texas A&M University); Suzanne McDermott (Seattle Children's Research Institution); Al Ivens (University of Edinburgh); Achim Schnaufer (University of Edinburgh); Sunil Kumar Sharma (Texas A&M University); Zachary Goodall (Texas A&M University); Xiuren Zhang (Texas A&M University); Jorge Cruz-Reyes (Texas A&M University)

Trypanosoma brucei has a complex life cycle requiring adaptation to very different hosts: procyclic form in the insect vector and bloodstream form in mammals. Rapid adaptation of the parasite during development includes changes in U-indel maturation of mitochondrial mRNAs. U-indel editing is catalyzed by a multi-complex holo-enzyme “the editosome” and directed by antisense guide RNAs (gRNAs) also encoded in mitochondria. One editosome component which we identified and termed the RNA Editing Helicase 2 Complex (REH2C) includes RNA Editing Helicase 2 (KREH2), KREH2-associated zinc-finger protein (KH2F1), and KH2F2. Recombinant KREH2 exhibits 3’-5’ ATP-dependent unwinding activity, and directly binds RNA and recombinant KH2F1, which also functions in editing. DExH/RHA helicases, including KREH2, have a characteristic C-terminal multidomain cluster that includes the catalytic domain. Additionally, KREH2 includes two N-terminal double-stranded RNA binding domains (dsRBDs). We have reported that overexpression of KREH2 WT or dominant negative (DN) with mutations: (a) “dead” helicase with inactive ATP binding site; or (b) in the dsRBD, induces a growth phenotype but the reason for this was unclear. Here, we will discuss new data examining these cell lines by biochemical techniques, RNA-Seq, and immunofluorescence, showing that KREH2 disregulation and DN mutants inhibit RNA editing, and disrupt interactions between protein and RNA components in editosomes. Our results align with other recent findings in our lab (discussed in a second abstract in the KMCB meeting) showing that REH2C normally controls the total number of edits, their fidelity, and remodeling of RNA targets in vivo.

34 Massive Upregulation of COII mRNA Editing Correlates with Alterations in RESC Protein and RNA Interactions During *T. brucei* Differentiation

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Twelve of the 18 mitochondrially encoded mRNAs in *T. brucei* must be post-transcriptionally modified by insertion/deletion of uridine residues (U-indel editing) to establish an open reading frame that is competent for translation on mitochondrial ribosomes. Although U-indel editing is essential throughout the life cycle, the extent to which specific mRNAs are edited varies in different life cycle stages. Here, we investigated changes in total and edited mRNAs in pleomorphic *T. brucei* during differentiation from long slender bloodstream form (BSF) to procyclic form (PCF). Most total and edited mRNAs increase modestly in stumpy forms and return to near slender BSF levels in PCF. In contrast, when stumpy BSF parasites are transitioned to PCF medium, there is a 20-fold increase in edited COII mRNA with no corresponding increase in the total COII mRNA abundance that is temperature-sensitive. This increase in edited COII mRNA is also reflected in vivo during tsetse fly midgut infections. RNA immunoprecipitation assays revealed that CCA-mediated differentiation triggers a dramatic increase in the association of COII mRNA with the RNA Substrate Binding Complex (RESC) during differentiation that is maintained in PCF parasites. We confirmed that the accessory protein, p22, is required for robust COII mRNA editing in pleomorphic PCF and for differentiation. Strikingly, the essential RESC protein, RESC13, dramatically decreased its association with p22 during differentiation. Altogether, from these data we propose a working model in which a decrease in temperature triggers an alteration in p22-RESC interactions that is permissive for editing of COII mRNA during differentiation to PCF.

35 Visualizing the process of variant surface glycoprotein shedding in response to trypanosome lytic factor

Kayla Leiss (Hunter College department of biological sciences); Alisha Rancho-Jensen (Hunter College department of biological sciences); Sophio Kirimlishvili (Hunter College department of biological sciences); Sara Fresard (Hunter College department of biological sciences); Jayne Raper (Hunter College department of biological sciences)

African trypanosomes are flagellated, extracellular parasites that infect humans and cattle causing African trypanosomiasis. Trypanosome lytic factor (TLF), a subset of high density lipoproteins (HDL), provides immunity to a species of trypanosomes, *Trypanosoma brucei brucei* in humans and some higher order primates through the osmotic lysis of the parasite. Trypanosomes are characterized by their coat of variant surface glycoproteins (VSG) which densely cover the plasma membrane of the parasite and allow it to evade the immune system through antigenic variation. VSGs are attached to the extracellular face of the plasma membrane by a glycosylphosphatidylinositol anchor (GPI) which is cleaved by GPI-phospholipase C under osmotic stress. The lytic component of TLF, Apolipoprotein L1 (APOL1), is a nonselective cation channel that induces trypanolysis through generation of an ionic and osmotic imbalance. Given that the VSG coat is shed during osmotic stress, we hypothesized that TLF-induced lysis would lead to the shedding of the VSG coat. To test this hypothesis, we fluorescently labeled the VSG coat with the lectin wheat germ agglutinin, bound to a fluorophore, and treated trypanosomes with HDL (TLF), to visualize the changes in VSG coat fluorescence using confocal microscopy. HDL (TLF) treatment resulted in a gradual decrease in fluorescence as the time of HDL (TLF) treatment increased. To better understand the mechanism of trypanosomes shedding the VSG coat, the next step will perform a similar immunofluorescence with GPI-PLC knockout parasites to evaluate the relationship that VSG shedding has with TLF-induced lysis.

36 Glycosomal Protein kinase A in *T. brucei*: a potential regulator of metabolism?

Kristina Malenica (Faculty of Biology, Ludwig-Maximilians-University Munich); Ignasi Forne (Biomedical Center, Ludwig-Maximilians-University Munich); Axel Imhof (Biomedical Center, Ludwig-Maximilians-University Munich); Frederic Bringaud (MFP (CNRS UMR-5234), University of Bordeaux); Michael Boshart (Faculty of Biology, Ludwig-Maximilians-University Munich)

Metabolic adaptation to changing nutrients, specifically carbon sources, is a key event in the host-parasite and vector-parasite interactions. *Trypanosoma brucei* rapidly consumes blood glucose when reaching the tsetse fly midgut. Glycerol might be transiently released during breakdown of the lipids in erythrocyte membranes after the blood meal, while amino acids are the main energy sources in the fly. Switching from glycolytic carbon sources that are metabolized in glycosomes to proline metabolized in the mitochondrium goes along with changes in number and protein composition of glycosomes. Signaling events involved in this regulation are essentially unknown to date. We found that a protein kinase A (PKA) catalytic subunits isoform (PKAC2) localizes to glycosomes in procyclic trypanosomes. Depletion of the glycolytic carbon sources increases PKA-mediated phosphorylation of a glycosomal substrate in vivo and several metabolic enzymes are regulated in abundance in a ∆pkac2 cell line. Interestingly, using a ± carbon source phosphoproteome we identified a member of the glycosomal import machinery as dominant PKA substrate. Crispr/Cas9-generated mutant lines and RNAi lines are currently used to evaluate the physiological role of carbon source depletion-induced PKA phosphorylation and thus in PKA-mediated metabolic adaptation.

37 Assessing Antigenic Distinction of Novel Mosaic VSGs found in *Trypanosoma brucei*

Kevin J Wang (JHSPH); Jaclyn Smith (JHSPH); Monica Mugnier (JHSPH)

T. brucei uses antigenic variation to switch its VSG coat, escaping host immune recognition. Most of the ~2000 VSG encoding genes are incomplete and must be accessed through recombination, creating new “mosaic” VSGs. Our lab developed VSG-AMP Seq, a targeted sequencing approach that allows characterization of mosaic VSGs. Using VSG-AMP Seq, we observed that VSG AnTat1.1 frequently recombines with EATRO1125 VSG-228 to form ~70 bp short, templated insertions throughout the gene. VSG-228 is a complete VSG that is highly similar to AnTat1.1. These mosaic VSGs seem to utilize short stretches of homology, creating insertions that are in frame. We examined if these mosaic VSGs are antigenically distinct from AnTat1.1. To that end, we created multiple transgenic Lister 427 parasite lines expressing different VSGs: AnTat1.1, VSG-228, or mosaic combinations of the two. Using FACS, we aim to determine if antibodies raised against AnTat1.1 are capable of binding AnTat1.1/VSG-228 mosaics. In a preliminary experiment, we observed that antibodies against AnTat1.1 are unable to bind VSG-228. From an orthogonal approach, we plan to immunize mice against VSGAnTat1.1 using live parasites and cure them with Berenil. Following a robust IgG response, we plan to challenge the mice with Lister 427 parasites expressing AnTat1.1, VSG-228, and AnTat1.1/VSG-228 mosaics. The survival of parasites within these mice will demonstrate whether antibodies generated against AnTat1.1 are protective against mosaic AnTat1.1 derivatives. These results will reveal the importance of short, templated insertions in antigenic variation and help define what protein changes are necessary to escape immune clearance.

38 *T. brucei* heterogeneity in the adipose tissue

Lara Lopez Escobar (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Sandra Trindade (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Mariana De Niz (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa); Milena Jakimovska Ozdemir (Acidabem University); Ugur Sezerman (Acidabem University); Luísa M. Figueiredo (Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa)

In a mouse infection, the adipose tissue is a major parasite reservoir of *Trypanosoma brucei*. In this tissue, parasites remodel their gene expression and activate fatty acid β-oxidation, suggesting that these parasites may use fatty acids as an external carbon source. Whether these changes affected parasite replication and whether they were homogeneous in the whole population remained unknown. Using proteomics and protein synthesis analysis, we found that the translation rate of the population of adipose tissue forms is 24% lower than parasites isolated from blood. Given that reduced protein synthesis is typically associated to reduced proliferation, next we measured proliferation rates at single cell level in these two tissues. We observed that the adipose tissue forms divide on average every 12hr, while bloodstreams divide faster (every 6hr). Surprisingly, we noticed that the range of doubling times is wider in adipose tissue forms (5 to 31hr) than in the bloodstream forms (4 to 11hr). Currently, we are using a single cell RNA sequencing (10x Genomics) to characterise the transcriptomic profile of individual parasites in the adipose tissue. Preliminary data suggests that adipose tissue is populated by a fast-growing subpopulation and a slow-growing subpopulation. Understanding cell-to-cell heterogeneity is important not only to uncover fundamental aspects of this parasite life cycle, but also to provide a potential new framework on disease relapse and drug treatment failure.

39 Phenotypic and genotypic characterisation of *Leishmania* (Viannia) *braziliensis* clinical isolates with variable susceptibility to AmB.

Laura Gonzalez (Universidad de los Andes); Carolina Vargas (Universidad Nacional de Colombia - Sede Bogotá); Samanda Aponte (Universidad Nacional de Colombia - Sede Bogotá); Aura Rodriguez (universidad del Tolima); Liliana Bonilla (Universidad Nacional de Colombia - Sede Bogotá); Marcela Parra-Muñoz (Universidad Nacional de Colombia - Sede Bogotá); Juan Matiz (Universidad Nacional de Colombia - Sede Bogotá); Maria P. Rodirguez (Universidad Nacional de Colombia - Sede Bogotá); Daniel Urrea (Universidad del Tolima); Joge Duitama (Universidad de los Andes); Maria Echeverry (Universidad Nacional de Colombia - Sede Bogotá)

Leishmania (V) *braziliensis* [L.(V)b.] is the parasite species most frequently associated with therapeutic failure for all treatment lines in cutaneous Leishmaniasis (CL) when compared with the other *L. Viannia* subgenera species. However, there is not a clear match between the genotypic changes observed in induced resistant parasites vs field isolates with low in vitro susceptibility to different pharmacological compounds. Here, 13 clinical isolates of L.(V)b. with few (~10, “early”) and 2 with many (>20, “late”) in vitro passages have been phenotypically characterised, in both promastigote and intracellular amastigote stages, and whole-genome sequence analysis was performed. Main findings support the idea of a variable phenotypic behaviour even within the same isolate early/late versions. Promastigotes in vitro doubling time in Schneider medium was estimated from 8.01 to 17.9 hours and AmB IC50 varied from 0.016 µg/ml to 0.207µg/mL. For amastigotes the AmB IC50 range observed was of 0.008 µg/ml - 0.244µg/ml. Percentage of in vitro infection to U937 cell line was calculated from 11.49% to 64%. Ploidy analysis showed that all isolates are diploid except for chromosome 31 and exhibited an increase in read depth of a fragment spanning about 60 kb on 13 genes on chromosome 34. One isolate displays the amplification of the LD1 region. De novo sequencing by Nanopore for early and late versions of the same isolate yielded highly contiguous assemblies including 8500 gene families. Comparative analysis of gene expansions revealed CNVs involving several genes that could be related to the observed changes in susceptibility to AmB.

40 Identification of *Trypanosoma cruzi* flagellar proteins in replicative stages

Madalyn M Won (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health); Barbara A Burleigh (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health)

The *Trypanosoma cruzi* flagellum is largely uncharacterized in all life stages, particularly in the parasite's non-motile intracellular amastigote stage. Amid growing evidence that the flagella of trypanosomatid species have important sensory roles, we predict that the *T. cruzi* amastigote flagellum may also function in environmental sensing or nutrient acquisition. Consistent with this suggestion, we have found that the *T. cruzi* amastigote flagellum is capable of movement and engages physically with mitochondria in infected host cells. With little basis to interrogate the potential functions of this understudied organelle, we have embarked on a proteome discovery effort based on proximity-dependent biotinylation and profiling of *T. cruzi* flagellar proteins by mass spectrometry. We generated *T. cruzi* lines that stably express TurboID targeted to the flagellum or cytosol and have profiled both epimastigotes (insect stage) and intracellular amastigotes (mammalian stage), and performed a comparative analysis of flagellar-enriched protein fractions. 78 proteins were identified as enriched in the flagellum in both life stages, 87 proteins only in the amastigote flagellar samples, and 185 only in the epimastigote flagellar samples. Of the candidates emerging as significantly enriched in the flagellar biotinylome of *T. cruzi* amastigotes, membrane-associated and soluble proteins (i.e., non-axonemal proteins) have been prioritized for validation and further study.

Poster Session C Abstracts

Friday 7 PM
September 16th 2022

41 TT5H An orphan, kinetoplastid-specific subunit of the Origin Recognition Complex?

Catarina Marques (University of Glasgow); Marcelo da Silva (University of Sao Paulo); Jose Batista (University of Glasgow); Calvin Tiengwe (Imperial College London); Richard McCulloch (University of Glasgow)

DNA replication is initiated at specific genomic loci termed origins, which are defined by the binding of a six-subunit Origin Recognition Complex (ORC). Though frequently described as highly conserved across eukaryotes, phylogenetic studies suggest many species may have lost one or more ORC subunits. In *Trypanosoma brucei*, four ORC subunits (ORC1/CDC6, ORC4, ORC5-like and ORC2-like) have been identified to date, all of which diverge considerably from their likely eukaryotic orthologues. The two remaining subunits, ORC3 and ORC6, remain elusive. Using a large group of publicly available genome and transcriptome datasets, we have run extensive sequence-based similarity searches, revealing Orc3 orthologues in all analysed Discoba lineages except for the kinetoplastids. Immunoprecipitation of multiple TbORC subunits resulted in the common identification of Tb1120 (Tb927.6.1120), and gel filtration analysis showed Tb1120 elutes in a complex of similar size to TbORC. Moreover, RNAi depletion resulted in growth arrest, cell cycle de-regulation, and abrogation of DNA replication. Sequence-based searches failed to provide evidence for a relationship between Tb1120 and any known ORC subunit, and orthologues of Tb1120 could not be found outside the kinetoplastids. However, de novo structure predictions suggest Tb1120 may have winged helix and AAA+ ATPase features found in ORC subunits. Our data suggest divergence of kinetoplastid ORC either through repurposing or replacement of at least one ORC subunit, an adaptation that has been accommodated by divergence of *T. brucei* ORC2 and ORC5 subunits. The potential purpose(s) of such restructuring of a core component of the DNA replication machinery remains unclear.

42 TT5K Mosaic VSGs Form as a Consequence of DNA Damage Repair in vitro and in vivo

Jaclyn E Smith (JHUSOM); Alex Beaver (JHUSOM); Shane Gilligan-Steinberg (JHBSPH); Sarah Sudlow (JHBSPH); Monica R Mugnier (JHBSPH)

Mosaic VSGs are crucial for *T. brucei* antigenic variation, the process where parasites change their surface antigen, a single VSG, to evade host antibody. There are ~2000 copies of VSG in the genome, consisting mostly of partial fragments and pseudogenes. Two or more VSGs, often incomplete themselves, combine to form novel VSGs known as mosaics. Mosaic VSG formation allows parasites to generate a nearly unlimited supply of new VSGs and is necessary for persistent chronic infection. Despite their importance, little is known about mosaic VSG formation or dynamics during infection. To this end, we developed VSG-Anchored Multiplex PCR Sequencing, a targeted sequencing approach to detect mosaic derivatives of a VSG of interest. Targeting VSG Antat1.1 in μMT mice, which lack B cells and never clear the initial AnTat1.1-expressing population, we observed patterns of recombination throughout AnTat, including numerous templated insertions consistent with DNA damage repaired through microhomology-mediated end joining (MMEJ). To investigate mosaic formation following DNA repair, we used an inducible Crispr/Cas9 system to introduce a double strand break within the N-terminus of AnTat1.1 in vitro. Remarkably, numerous AnTat1.1 mosaics formed immediately adjacent to the break site, with the same donor VSGs and patterns we observed in vivo. Accumulation of insertions over time, or insertions in particular positions, may be sufficient to produce novel antigens. These experiments demonstrate, for the first time, that mosaic VSGs can form as a consequence of DNA damage. Importantly, our observations and approach allow us to elucidate a previously inaccessible feature of antigenic variation.

43 TT5L A single pseudouridine on rRNA matters: A specific effect of rRNA modification on translation in *Trypanosoma brucei*

K. Shanmugha Rajan (Bar-Ilan University); Hava Madmoni (Bar-Ilan University); Saurav Aryal (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Beathrice Galili (Bar-Ilan University); Katherina Adler (Bar-Ilan university); Ron Unger (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Shulamit Michaeli (Bar-Ilan university)

Differential pseudouridine (Psi) modification on rRNA, snRNA, tRNA was suggested by us to play a role in the parasite transition between its two hosts (Chikne et al., Sci.Rep. 2016, Rajan et al., Nucleic Acids Res. 2019, Rajan et al., J. Biol. Chem. 2022). Here we used several mapping methods of pseudouridine such as Psi-seq, HydraPsiSeq and nanopore RNA sequencing to quantitatively determine changes in pseudouridine on rRNA of procyclics (PCF) and bloodstream (BSF) parasites. The hypermodified sites in BSF were localized to the H69 domain present in the inter-subunit bridge, A-site finger domain, and near the decoding center. To investigate the role of these hypermodified sites and their implications, we performed CRISPR-Cas9 knock-out of the snoRNA TB11Cs6H1 guiding Psi-530 on H69. The growth of sKO was compromised and quantitative mass-spectrometry identified changes in the level of only a subset of proteins; most are developmentally regulated. Add back of TB11Cs6H1, but not a defective mutant was able to reverse the sKO phenotype. To investigate the mechanism by which a single modification affects translation, rRNA structure was determined by dimethyl sulfate (DMS) mapping in vivo and changes in the structure of H69 and other rRNA domains, were observed. Analysis of 80S ribosomal protein composition detected major reduction the level of eS12. We propose that the change in Psi-530 affected the secondary structure of the rRNA, leading to dislodging of eS12 and producing ribosomes depleted of this protein. These “altered” ribosomes result in specific changes in translation. Thus, the differential level of Psi modification in the two life stages may contribute to stage-specific translation regulation during cycling between the hosts.

44 Using yeast surface display to identify phosphoinositide-binding proteins in trypanosomes

Mengjin Gao (McGill University); Andressa Brito Lira (McGill University); Tamara Sternlieb (McGill University); Mira Loock (McGill University); Igor Cestari (McGill University)

Trypanosoma brucei Phosphoinositide (PI) signaling plays a central role in the control of immune evasion and life stage development in trypanosomes. The PI system entails ~30 proteins involved in the synthesis, transport, and modification of soluble inositol phosphates (IPs) or membrane-bound PIs via phosphorylation or dephosphorylation. PI kinases and phosphatases and their substrates and products have been identified in the plasma membrane, cytosol, and organelles including in the nucleus. Dissecting the regulatory function of the PI signaling system requires identifying proteins that interact with IPs or PIs. However, a limited number of proteins have known domains that bind phosphoinositide metabolites, including pleckstrin homology or FYVE domains. We propose using yeast surface display (YSD) to identify proteins that bind to IPs and PIs. We generated a *Trypanosoma brucei* genome-wide library for YSD. The library represents ~7-fold of the parasite genome and has DNA fragments ranging from 0.5 to 3Kb. Nanopore sequencing showed that the library includes every parasite gene and computational analysis predicted the expression of ~180,000 distinct polypeptides, including complete proteins or fragments. Western blot also confirmed the expression of library proteins in yeast. We are screening the YSD library to identify proteins that bind to IPs or PIs, e.g., PI(4,5)P2 and Ins(1,4,5)P3. Our work involves magnetic-activated cell sorting followed by nanopore sequencing of library sequences from selected yeast. This work will shed light on PI signaling and regulation in trypanosomes.

45 Cleavage of *Trypanosoma brucei* FoF1-ATP synthase alpha subunit: A rare attribute

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While the structure and composition of the catalytic F1 domain of the mitochondrial ATP synthase was considered to be highly conserved throughout eukaryotes, the crystal structure of the enzyme from the early diverging protist *Trypanosoma brucei* revealed several unique attributes. This includes the proteolytic maturation of the alpha subunit at two internal cleavage sites that expels an octapeptide. This generates mature N- and C-terminal alpha peptides that are both incorporated into the functional FoF1-ATP synthase. Since this enzyme is essential for the parasite, we aim to decipher the biological significance of this alpha subunit proteolysis. Bioinformatic approaches suggest that the recognition sequence for both proteolytic events is retained primarily within the octapeptide. This is supported by the observation of a proteolytic resistant *T. brucei* alpha in which the octapeptide was replaced with the FLAG epitope. Importantly, this alpha FLAG mutant is only partially incorporated into assembled FoF1-ATP synthase complexes. Planned in vitro activity assays of enzymes containing an uncleaved alpha will decipher if alpha proteolysis is necessary for proper F1 function. Since the expression levels of alpha FLAG proteins are limited, we are currently analyzing the ability of next generation alpha octapeptide mutants to rescue alpha RNAi cell lines. In addition, in vitro FRET assays are being implemented to identify the protease(s) responsible for alpha proteolysis. Interestingly, subcellular fractionations and immunofluorescence microscopy indicate that the octapeptide can act as a mitochondrial targeting sequence for the mouse cytosolic dihydrofolate reductase, indicating that the first octapeptide cleavage could occur in the cytoplasm.

46 Heme Homeostasis at the *Leishmania*-Host Interface

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Visceral Leishmaniasis is a devastating disease caused by the protozoan parasite *Leishmania donovani*. The clinical manifestations of visceral Leishmaniasis include anemia, enlargement of spleen and liver, and death if left untreated. Once a mammalian host becomes infected with *L. donovani* via phlebotomine sand fly bite, parasites invade host reticuloendothelial macrophages. Although these parasites replicate uncontrollably once they have entered a host, they have a weakness: *L. donovani* lack the ability to synthesize heme, an essential cofactor for a diverse range of biological processes. *Leishmania* acquire heme from their host via LHR1, a homolog of mammalian heme transporter HRG1. Although *Leishmania* growth and survival is reliant on host heme, the relationship between host heme status and parasite fitness has remained underexplored. Here we show that host heme availability directly impacts parasitemia and the HRG1-LHR1 axis plays a central role in the host-parasite competition for heme. We found that mice with mutations in the heme homeostasis pathway show striking differences in both parasite load and splenomegaly, which challenge the current paradigm that these characteristics are correlated. Conversely, perturbations to the parasite heme acquisition pathway significantly affect parasitemia. Taken together, our results demonstrate that heme is critical for host nutritional immunity. We plan to identify key regulators of heme acquisition through genetic and cell biological perturbation of the parasite and its host macrophage and generating *L. donovani* expressing a novel dual reporter construct to identify differences in the kinetics of infection in vivo. Uncovering the role of essential nutrients in this potentially fatal condition could elucidate potential parasite-specific therapeutic targets that would limit host toxicity and improve quality of life.

47 Single-cell RNA-sequencing: When things go wrong…

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Single-cell RNA-sequencing is a powerful tool to study cell-to-cell heterogeneity and many other cellular properties. However, library preparation and data analysis for scRNA-seq is challenging and can lead to a long list of artifacts. Here, we report from our experience in using plate-based scRNA-seq approaches such as Smart-seq2, Smart-seq3express and mcSCRB-seq, as well as the droplet based 10x Genomics technology, to measure variant surface glycoprotein (VSG) expression. Importantly, some of the listed approaches led to significant ‘barcode-hopping’, resulting in the assignment of transcripts to the wrong cells. The consequence was an apparent loss of mutually exclusive VSG expression when the cells were in fact still expressing a single VSG. By sharing our experiences, we hope to inform others about the pros and cons of the different scRNA-seq approaches at a time when a growing number of kinetoplastid research groups begin to establish this technology in their labs.

48 Visualizing metacyclic VSG coat synthesis in live trypanosomes

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In the salivary glands of the tsetse fly the VSG coat must be synthesized de novo in cells developing into infectious metacyclic forms. To monitor this process in real time in live trypanosomes, we used an inducible in vitro system based on overexpression of Cold Shock Domain protein 1 in procyclic cells for triggering metacyclic VSG coat synthesis. To visualize VSG proteins in live cells we developed a chimeric VSG gene (N- and C-terminal domains for VSG-531 and GPI-anchor signal for VSG-397) tagged with mNeonGreen inserted immediately upstream of the GPI-anchor signal. The construct was integrated into the VSG-397 metacyclic expression site by replacing the original VSG-397 CDS. By using fluorescence microscopy, long-term live-cell imaging, immunoelectron microscopy and expansion microscopy, we observed that the fluorescently tagged VSG is properly delivered to the cell surface. Additionally, we confirmed a previous finding [Tetley et al., 1987] describing a very strong initial flagellar membrane enrichment of newly synthesized metacyclic VSG. We used the tagged VSG experimental system in a low-throughput small molecule screen (33 phosphatase and 53 protease inhibitors) in search for molecules that interfere with VSG coat synthesis. One of the hits, NapSul-Ile-Trp-CHO, a cathepsin L inhibitor, caused the localization of large fraction of the fluorescent VSG to an enlarged lysosomal compartment and a delay in the trafficking of the protein to the flagellar membrane and the cell body surface. This screening approach has the potential to be developed into a high-throughput microscopy assay for identifying molecules that inhibit VSG coat synthesis.

49 TriTrypDB: Free online informatic tools for kinetoplastid biologists

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TriTrypDB (https://TriTrypDB.org) is a free online resource enabling browsing, querying, and sophisticated mining of genomic-scale datasets. TriTrypDB is a component of The Eukaryotic Pathogen, Vector and Host Informatics Resources (VEuPathDB.org) that integrates genomic, transcriptomic, proteomic, epigenetic, population diversity, phenotypic, and other types of data for eukaryotic microbes (pathogenic & nonpathogenic, free-living & parasitic), as well as the hosts and vectors of human disease. TriTrypDB includes genomic and large scale omics data from the major human pathogenic kinetoplastida (*Leishmania*, African and American Trypanosoma). A user-friendly web interface with embedded bioinformatics tools supports in silico experiments leveraging public data, along with private datasets uploaded by the user into “My Workspace” via the VEuPathDB Galaxy interface. With TriTrypDB, you can browse genomes and examine gene record pages, create searches to mine Omics-scale datasets, annotation, and the results of automated analyses (protein domains, orthology predictions from OrthoMCL.org, metabolic pathways, etc.) Expert knowledge of genes, phenotypes, publications, and other gene-centric data may be captured as User Comments, via bulk data submission, or through the integrated Apollo annotation platform. New datasets can be nominated for integration by emailing the TriTrypDB help desk or via social media. During the KMCB meeting stop by our help desk staffed during all poster sessions to ask questions, discuss your data or participate in the “Got Parasites?” user comment campaign. Email us any time with questions and suggestions: help@VEuPathDB.org. VEuPathDB is one of two NIAID-supported Bioinformatics Resource Centers, and receives additional support from the Wellcome Trust and the Bill & Melinda Gates Foundation.

50 Establishing a CRISPR-dCas visualization system to study genome architecture changes in *T. brucei*

Prateek Yadav (LMU Munich); Richard Wheeler (University of Oxford); T. Nicolai Siegel (LMU Munich)

Trypanosoma brucei causes African trypanosomiasis and uses antigenic variation to evade the host immune system. Antigenic variation refers the pathogen's ability to switch the antigens exposed to the immune system, leading to chronic infection. Recent findings from our lab suggest that the spatial proximity of different antigen-coding genes (VSGs) is essential for a successful switching event. To verify this, we are establishing a CRISPR-dCas imaging system targeting several VSGs and their interacting partners in an effort to visualize the genomic architecture during an antigenic switch in vivo. In order to image a genomic locus, the dCas protein needs to be nuclear and well regulated. Our immunofluorescence assay findings showed that dCas with the monopartite NLS described from C terminus of the La protein, the most commonly used nuclear localization sequence (NLS) in *T. brucei*, did not localized to the nucleus. We hypothesize that this is either due to the NLS or the overall protein size. Therefore, we tested a variety of NLSs identified in TrypTag at both termini of different dCas proteins fused to mNeonGreen or the epitope tags Ty1 or HA. We observed that some protein combinations like dCas::mNG::10XGS::NLS localized to the nucleolus with or without sgRNA. Our findings suggest that the protein size and NLS are crucial for traversing the nuclear pore. However, high protein levels might prevent dCas to stay in the nucleoplasm. We will discuss the different protein-NLS combinations and difficulties in setting up a dCas-based imaging system.

51 A branched chain amino acids transporter in the procyclic form of *Trypanosoma brucei*

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Through its life cycle, *Trypanosoma brucei* has to adapt to changing nutrient availability. In order to do so, transporters play an important role by facilitating the uptake of nutrients as for example amino acids. Genome analysis from Jackson et al., (2007) identified 17 loci for amino acid transporter genes, including Tb427.08.8300 (AAT10-2), which is further studied in our lab. We could show that AAT10-2 is essential in the procyclic form (PCF) of *T. brucei*, demonstrated its preference for branched chain amino acids (BCAA) as substrate and identified several phosphorylated residues in the N-terminal domain.

52 From TrypTag to LeishTag: Conservation of localisation?

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Leishmania and *Trypanosoma* are rightfully viewed as closely related parasites; however, these species diverged 100s of millions of years ago and have known differences in life cycles, with specific adaptations for pathogenicity. How much of their molecular cell biology is closely related? TrypTag (tryptag.org), the recently completed genome-wide localisation resource has provided a detailed view of the position of the vast majority of proteins within a *T. brucei* cell. Building on the success of this project and the CRISPR/Cas9 revolution of *Leishmania* genetics, we have initiated the LeishGEM project. LeishGEM is a collaborative project using high-throughput reverse genetics to determine protein subcellular localisation of tagged proteins and function through fitness phenotyping of deletion mutants in *Leishmania mexicana*. We will use an endogenous tagging approach to localise proteins primarily with either no ortholog (2513 *L. mexicana* genes do not have a reciprocal best BLAST hit in *T. brucei*) or high sequence divergence (692 have <20% identity to their *T. brucei* ortholog). As of June 2022 we have localisation data for 326 *L. mexicana* proteins in the promastigote and amastigote. We can now start determining in which organelles/structures lineage-specific adaptations for the independently-evolved mammalian parasitism have occurred.

53 Identification of RNA binding targets and protein interactors of the *Trypanosoma brucei* RNA-binding protein 7B during metacyclogenesis

Saúl Rojas Sánchez (Yale School of Public Health); Christian Tschudi (Yale School of Public Health)

Overexpression of the RNA-binding protein 6 (RBP6) in procyclic trypanosomes triggers a developmental pathway that culminates with the production of infectious and quiescent metacyclics. Among 24 recently identified regulators of this process, we found RBP7A and RBP7B, components of the stumpy induction factor (SIF) signaling pathway. Despite being ~85% identical at the protein level, downregulation of each transcript during RBP6 expression revealed distinct roles in metacyclogenesis. Downregulation of RBP7A enhanced metacyclic production with a concomitant appearance of quiescent cells aberrantly arrested outside of G1/G0. In contrast, RBP7B downregulation prevented the large transcriptional changes induced by RBP6 and the appearance of metacyclics. To determine if either RBP7 protein was found in a complex, we performed ultracentrifugation on sucrose gradients. While RBP7A remained in the soluble fraction, RBP7B co-sedimented with the light polysome fraction. Both copies of RBP7 have a mass of ~13 kDa. However, RBP7B migrated between the 66 and 146 kDa markers in native gels, but RBP7A was found below 66 kDa. These observations strongly suggest that RBP7B is present in a higher-order complex. To investigate the RBP7B interactome, we performed BioID. Mass-spectrometry analysis showed several possible RBP7B interactors with at least one peptide present in three biological replicates and absent in the negative control. Finally, to determine RBP7B RNA binding targets, we performed seCLIP. We identified 3,131 significant binding regions (log2 fold change > 3; $p < 0.01$) in 2,323 transcripts. Around 68% of them were located in coding sequences. MEME analysis of RBP7B targets revealed the motif UUGCUG with an excellent confidence value (E = 3.3 e-225).

54 TLF3: Identification of a unique pre-beta high density lipoprotein that contributes to immunity against African Trypanosomes

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African Trypanosomiasis is a disease caused by the African Trypanosoma family of bloodborne parasites. Humans and some non-human primates are protected against most species of trypanosomes due to an immunity complex called Trypanosome Lytic Factor (TLF). TLF is a specialized High-Density Lipoprotein (HDL), that carries a lytic cation channel-forming protein (Apolipoprotein-L1 (APOL1)), and a ligand (Haptoglobin-related protein (HPR)), which binds to parasite receptors enhancing uptake into the parasite. There are two TLF HDL complexes in blood: TLF1 (~500 kDa) and TLF2 (~1,200 kDa). How these TLFs are formed is unknown. To fully characterize all possible TLF species, we used anti-APOL1 affinity chromatography to purify all complexes containing APOL1 from human serum. Size exclusion chromatography by FPLC, using multiple columns, allowed us to further separate the complexes by size. Both TLF1 and TLF2 were isolated, as well as a third complex that is 180 kDa. Western blotting of the fractions confirmed co-elution of APOL1, HPR, and Apolipoprotein A1, a protein found in all HDLs, suggesting this complex is a distinct HDL. Lysis assays showed this complex lyses trypanosomes. Immunoprecipitation confirmed that all three proteins are on the same complex. We suggest that this TLF complex, TLF3, is a pre-beta HDL, based on its size and density. We hypothesize that all three proteins are assembled together in/on the hepatocyte with minimal but sufficient lipids to prevent clumping of the apolipoproteins. The TLF3 complex is released into the blood and matures into TLF1 by collecting cholesterol and phospholipids as it circulates through the body.

55 APEX2 proximity labelling for mapping mRNP-NUP interactions during nuclear export

Siqi Shen (Charles University); Johanna Odenwald (University of Würzburg); Bernardo Gabiatti (University of Würzburg); Susanne Kramer (University of Würzburg); Martin Zoltner (Charles University)

Unlike opisthokonts, trypanosomes transcribe mRNAs as long polycistrons, which are co-transcriptionally processed by trans-splicing and are lacking the mRNA export checkpoint that prevents immature mRNAs from starting nuclear export. We set out to leverage these unique features to study mRNA export in molecular detail and map the interactions between messenger ribonucleoprotein particles (mRNPs) and nucleoporins (NUPs). To capture these highly dynamic, transient interactions we can employ an “mRNA trap”, by biochemical inhibition of transcription or splicing, to trap mRNA in transit within the nuclear pore in combination with proximity labeling techniques. To this end, we have established APEX2 in *Trypanosoma brucei*, a proximity labelling technique for RNA and protein, relying on an ascorbate peroxidase that can be fused to the protein of interest and converts biotin-conjugated aromatic probes to a short-lived radical to react with RNA and proteins. We used the two *T. brucei* poly-A binding proteins (PABPs), which interactomes are well characterized, as a testbed and devised a strategy for efficient labelling and subsequent purification. PABP2 localises to stress granules upon starvation and granules at the nuclear periphery upon splicing inhibition. Significantly, in situ imaging via a fluorescent streptavidin probe traces biotin to these distinct structures, indicating high spatial labelling selectivity. We are currently targeting the first nucleoporin candidates for APEX2 proximity labelling, aiming to create a map of interactions between the nuclear pore proteins and mRNPs in transit with high spatial resolution.

56 *Trypanosoma brucei* TEL2 suppresses VSG switching

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Trypanosoma brucei is a unicellular protozoan parasite that causes African sleeping sickness in humans and nagana in cattle. *T. brucei* can evade the host immune response through antigenic variation, where it regularly switches to express different VSGs, its major surface antigen. VSGs are expressed exclusively from subtelomere loci in a strictly monoallelic manner, and we have shown that many telomere proteins play critical roles in VSG regulation. TEL2 was first identified in *Saccharomyces cerevisiae* as a telomere length regulator. A TEL2 mutation caused significant telomere shortening in budding yeast. However, different TEL2 homologs in yeast, worm, and mammals have different functions. Mammalian TEL2 has been shown to regulate the protein stability of PI3 kinase related kinases (PIKKs), which are involved in multiple signaling transduction pathways such as DNA damage repair, cell cycle checkpoint, and mRNA degradation, etc. We have identified the *T. brucei* TEL2 homolog and generated inducible TbTEL2 RNAi strains. We found that TbTEL2 is essential for cell proliferation. Particularly, a transient depletion of TbTEL2 significantly increases the VSG switching frequency. *T. brucei* has ATM, ATR, and several TOR homologs, which are PIKKs and play important roles in DNA damage repair and cell growth. In addition, *T. brucei* ATR has been shown to influence the VSG switching. Therefore, to investigate how TbTEL2 regulates VSG switching, we will test whether TbTEL2 helps maintain stability of these PIKKs. We have tagged TbTOR1 in the TbTEL2 RNAi cells and observed significant decrease in the TbTOR1 protein level after depletion of TbTEL2 by RNAi. Whether protein levels of other PIKKs are affected by TbTEL2 is currently under investigation. We are also investigating the underlying mechanisms of how TbTEL2 regulates PIKK levels.

57 *Trypanosoma carassii*, a model for whole host interaction studies.

Sarah Gaëlle Monic (University of Cambridge, Biochemistry department); Maria Forlenza (Wageningen University and Research); Mark Carrington (University of Cambridge, Biochemistry department)

Trypanosoma carassii is a freshwater fish parasite that infects a variety of cyprinids (carp family). The prevalence approaches 100% including in fish farms. Here, the procedures for long term culture and transgenesis of *T. carassii* are described as the first step in developing a model to study host-pathogen interaction in zebrafish. We show that *T. carassii* can be genetically modified using approaches developed in *T. brucei* and these have been used to make *T. carassii* cell lines expressing mNeonGreen and Ruby3 fluorescent protein transgenes driven by either RNA pol II or RNA pol I. The cell lines will be used to infect transparent zebrafish larva and will enable the tracking of all trypanosomes infecting a host. The response of the fish immune system will be visualised during infections of zebrafish lines with marked immune cell lineages. Together, these experiments will answer long standing questions about tissue tropism, sites of proliferation and extravascularisation, and how the host immune system responds in different tissues.

58 Track & trace: using dual-colour imaging to visualise transport in the beating flagella of *Leishmania mexicana*

Sophie Gray (University of Oxford); Richard Wheeler (University of Oxford)

Intraflagellar transport (IFT) is the bi-directional transport of proteins on IFT trains necessary for building flagella, including in *Leishmania mexicana*. *L. mexicana* promastigotes possess a 9+2 motile flagellum which is vital for normal life cycle progression. Since IFT discovery nearly three decades ago, IFT train movement has essentially exclusively been analysed in cells with flagella immobilised either mechanically or by mutations. To visualise IFT train movement in actively beating flagella we developed high framerate dual-colour fluorescence microscopy, using a triple mNeonGreen-tagged IFT protein (3×mNG::IFT172) and either red light phase contrast or mCherry-tagged flagellar proteins. IFT is visible in the green channel and the flagellum can be traced using the red channel, allowing simultaneous capture of flagellar bending and IFT train movement. Using this microscopy approach, we measured anterograde and retrograde IFT train speed in free moving flagella undergoing different beat types, in comparison to mechanically immobilised flagella. IFT trains may experience steric hinderance from other axoneme-associated complexes and presumably compete for ATP with the motor proteins driving axoneme beating. Therefore, we also measured IFT train speeds in distal docking complex (dDC) 1, dDC2, dynein light chain 1, outer dynein arm beta and radial spoke protein 4/6 deletion mutants with defective beats. We showed that IFT train speed is reduced by mechanical flagellum immobilisation and in beating flagella IFT train speed is independent of beat type. IFT train speed is also increased in mutants with defective, but still motile, flagella. Together this shows the importance of investigating IFT in beating flagella.

59 RESC8 and RESC14 cooperate to mediate RESC function and dynamics during trypanosome RNA editing

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Uridine insertion/deletion RNA editing is unique to kinetoplastid organisms and essential for their survival and virulence. This process requires the action of several multiprotein complexes and small trans-acting RNAs called guide RNAs. The RNA editing holoenzyme comprises three dynamically interacting complexes: RECC (RNA Editing Catalytic Complex), RESC (RNA Editing Substrate Binding Complex), and REH2C (RNA Editing Helicase 2 Complex). Previous studies in *Trypanosoma brucei* showed that RESC components, RESC14 and RESC8, are critical in maintaining protein-protein and protein-RNA interactions within the editing holoenzyme. To further clarify the role of these proteins in RNA editing, we performed high-throughput sequencing of A6 mRNA in RESC14, RESC8, and RESC13 RNAi cells, and compared the phenotypes of these knockdown cell lines. We measured a highly significant overlap between exacerbated pause sites arising on A6 mRNA in cells depleted of RESC14 and RESC8, suggesting an overlapping function between these proteins during editing progression. To probe the biochemical basis of the RESC14/8 interdependence, we performed Native PAGE analysis and showed that RESC8 is not incorporated into large RNA-containing complexes in the absence of RESC14. Native PAGE also showed that RESC14 is needed for incorporation of other RESC factors into large RNA-containing complexes, but is dispensable for RECC, KREH1 and REH2C complex formation. To better understand the interaction of RESC14 and RESC8, co-immunoprecipitation assays were performed and identified an RNA inhibited interaction between RESC14 and RESC8. Together, our findings suggest that RESC14 and RESC8 rely on each other for necessary protein and RNA rearrangements during editing progression.

60 Identifying telomeric expression site protein interactions at peptide resolution using cross-linking and mass spectrometry

Tony Isebe (McGill University); Igor Cestari (McGill University)

Trypanosoma brucei evade host antibody response by antigenic variation, periodically switching the expressed variant surface glycoprotein (VSG). *T. brucei* have ~2,500 VSG genes and pseudogenes; however, they only express one VSG gene at a time from one of the 20 telomeric expression sites (ESs). We previously identified a 0.9 MDa Telomeric Expression Site protein Complex (TESC) that functions in VSG silencing via a phosphoinositide-regulatory system. The TESC includes the enzyme phosphatidylinositol 5-phosphatase (PIP5Pase), repressor activated protein 1 (RAP1), DNA/RNA-binding proteins, nuclear lamina proteins, and protein kinases and phosphatases and appears to be resistant to nucleases. To understand the composition and organization of the TESC, we used a combination of in vivo chemical crosslinking, affinity purification, and mass spectrometry and analyzed the interactions in bloodstream and procyclic forms. Our data identified most of the previously identified TESC proteins with crosslinked peptides at a 1% false discovery rate. Dozen additional proteins were identified crosslinked to TESC proteins revealing an extensive network of telomeric ES protein interactions. Importantly, the data highlights interactions with nuclear lamina proteins suggesting a role of TESC in the compartmentalization of silent ESs near the nuclear periphery. The association of splicing factors with TESC also suggests RNA processing or compartmentalization of the transcribed ES. The results indicate direct interactions of the TESC at peptide resolution, including protein domains involved. Additional work will explore changes in the TESC interactions associated with VSG silencing and activation. Our findings yield insights into the composition and topology of the TESC in trypanosomes.

61 High-throughput scRNA-seq in *Trypanosoma brucei* with Smart-seq3express

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To date, there are no scalable methods that provide both low cost and high sensitivity single-cell RNA experiments in African trypanosomes. Yet, with an increasing number of researchers that focus on molecular events at the single-cell level, there is a clear need for such methods. One of the scRNA-seq methods that are widely used nowadays is a plate-based Smart-Seq2 technology. This method provides full coverage and is more flexible in terms of running different conditions simultaneously compared to droplet-based methods such as 10xgenomics. However, although Smart-seq2 technology provides higher sensitivity compared to other scRNA-seq methods, it is not suitable for large-scale scRNA-seq experiments because of a very high cost per cell. In addition, the absence of unique molecular identifiers (UMIs), makes accurate RNA quantification impossible. Smart-seq3 is an improved version of Smart-seq2 with higher sensitivity and incorporation of UMIs at the 5’ end of the read, yet costs remain high. Here we report our efforts to implement Smart-seq3express in *Trypanosoma brucei*, an improved and miniaturized version of Smart-seq3 that allows for even higher throughput with less manual handling at much lower cost per cell. In this latest protocol the working volume is on nanoliter scale which considerably cuts the costs of the previous methods and allows to process hundreds of samples (single cells) at once. As with Smart-seq2 and 3, the workflow involves oligo-dT priming, reverse transcription followed by template switching, preamplification of cDNA, tagmentation by Tn5 and library construction. In *T. brucei* mRNA processing involves adding a common spliced leader sequence at the 5’ end of each transcript through trans-splicing. When adapting Smart-seq3express to *T. brucei*, we took advantage of this feature, and developed a Smart-seq3 derivate that uses the common splice leader sequence as an alternative to the template switching step in the regular Smart-seq3 protocol. This modification makes the workflow more efficient.

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Author Index

A

Adler, Katherina · 22, 40, 93, 153
Aelmans, Marianne · 33, 127
Afasizhev, Ruslan · 24, 98
Afasizheva, Inna · 24, 98, 100
Afrin, Marjia · 27, 106
Ai, Hui-wang · 40, 156
Aibara, Shintaro · 16, 32, 74, 122
Akiyoshi, Bungo · 15, 69
Almeida, Igor C. · 18, 35, 36, 76, 77, 130, 140
Alvarez-Cabrera, Ana Lucia · 24, 98
Ammar, Mufida · 19, 81
Amunts, Alexey · 16, 32, 74, 122
Anderson, Edward A · 36, 135
Aponte, Samanda · 38, 148
Aryal, Saurav · 22, 40, 93, 153
Asiki, Hannah A · 36, 135
Asseri, Jamal · 32, 123

B

Barcons-Simon, Anna · 21, 84
Barnes, Chloe · 19, 80
Bart, Jean-Mathieu · 13, 31, 60, 114
Bartholomeu, Daniella · 22, 89
Batista, Jose · 22, 40, 91, 151
Beaver, Alex · 22, 27, 40, 92, 102, 152
Beltran, Miguel A. · 18, 77
Beraldi, Dario · 28, 107
Berenguer Antunes, Luisa · 13, 31, 62, 116
Beznosková, Petra · 15, 65
Billington, Karen · 41, 162
Billington, Neil · 35, 131
Black, Jennifer A · 36, 138
Blum, Carina · 30, 112
Blunk, Torsten · 30, 112
Bobb, Bryce · 27, 102
Bonilla, Liliana · 38, 148
Boshart, Michael · 37, 145
Bowman, Natalie · 36, 139
Braune, Silke · 32, 126
Bravo Ruiz, Gustavo · 9, 50
Bregy, Irina · 9, 48
Briggs, Emma M. · 12, 22, 28, 35, 57, 89, 107, 133
Bringaud, Frederic · 18, 37, 79, 145
Brito, Carlos R. · 18, 77
Budzak, James · 27, 105
Burchmore, Richard · 32, 123
Burleigh, Barbara A · 9, 18, 38, 45, 76, 149
Butter, Falk · 12, 18, 21, 30, 35, 58, 79, 88, 111, 134

C

Calderaro, Salvatore · 35, 132
Camara, Aïssata · 13, 31, 60, 114
Campagnaro, Gustavo Daniel · 33, 128
Campbell, Paul C · 35, 41, 131, 158
Carnes, Jason · 24, 36, 99, 137
Carrington, Mark · 15, 42, 67, 167
Carruthers, Ross · 28, 107
Carvajal, Elisa · 35, 130
Cassidy, Andrew · 16, 31, 71, 119
Cayla, Mathieu · 12, 55
Cestari, Igor · 13, 27, 31, 40, 62, 103, 116, 154, 170
Chauhan, Prashant · 16, 32, 74, 122
Chen, Runpu · 24, 42, 97, 169
Choi, Junyong · 13, 31, 63, 117
Coelho, Joana · 12, 30, 59, 113
Cohen-Chalamish, Smadar · 15, 22, 40, 66, 93, 153
Colomé-Tatché, Maria · 15, 41, 68, 157
Cosentino, Raúl O. · 15, 41, 42, 68, 157, 171
Crilly, Nathan P · 27, 102
Crouch, Kathryn · 22, 89
Cruz, Angela K. · 16, 31, 33, 70, 118, 128
Cruz-Reyes, Jorge · 24, 37, 96, 142

D

D'Ordine, Alexandra · 35, 131
da Silva, Marcelo · 22, 40, 91, 151
Damasceno, Jeziel · 22, 28, 89, 107
Dawson, Neil · 19, 80
de Graffenried, Christopher L · 35, 41, 131, 158
De Niz, Mariana · 12, 30, 59, 113, 147
Debler, Erik W. · 21, 27, 86, 88, 104
Debus, Andrea · 35, 134
De-koning, Harry · 32, 123
Dellinger, Virginia · 19, 81
Delzell, Stephanie B · 22, 90
Dewar, Caroline · 33, 127
Dobramysl, Ulrich · 41, 162
Dobrynin, Atai · 15, 41, 68, 157
Doleželová, Eva · 18, 78
Dong, Gang · 9, 49
Doniger, Tirza · 15, 22, 40, 66, 93, 153
Drouaud, Arthur · 13, 31, 61, 115
Dubey, Ashutosh P · 24, 97
Duitama, Joge · 38, 148
Dujeancourt-Henry, Annick · 13, 31, 60, 114
Dumoulin, Peter C · 18, 76
Durante, Ignacio M. · 15, 65
Durrani, Hina · 19, 36, 82, 136

E

Echeverry, Maria · 38, 148
Eden, Amelie · 15, 67
Eisenhuth, Nicole · 35, 134
Ellis, Cameron C · 18, 76
Eloiflin, Roger Junior · 13, 30, 60, 114
Engstler, Markus · 9, 12, 30, 45, 56, 112
Erben, Esteban · 21, 87
Erhard, Florian · 15, 68
Estevao, Igor · 18, 35, 76, 77, 130

F

Fant, Courtney · 19, 81
Farani, Priscila · 18, 35, 36, 77, 130, 140
Faria, Joana RC · 27, 104
Fathallah, Nadin · 19, 80
Figueiredo, Luísa M · 12, 13, 27, 30, 38, 54, 59, 102, 113, 147
Forlenza, Maria · 42, 167
Forne, Ignasi · 37, 145
Forteath, Jordan Alexander · 9, 50
Fradera Sola, Albert · 12, 30, 58, 111
Franco, Cláudio A · 13, 30, 59, 113
Fresard, Sara · 37, 41, 144, 164
Frisbie, Victoria · 21, 86, 88

G

Gabiatti, Bernardo · 15, 37, 42, 67, 141, 165
Gabriel, Heloise · 9, 49
Gahura, Ondřej · 16, 32, 74, 122
Galili, Beathrice · 15, 22, 40, 66, 93, 153
Gao, Mengjin · 40, 154
Gaurav, Amit Kumar · 27, 106
Gendrin, Claire · 36, 137
Gilligan-Steinberg, Shane · 22, 40, 92, 152
Gilman, Robert · 36, 139
Girasol, Mark · 28, 107
Glover, Lucy · 13, 31, 60, 114
Gluenz, Eva · 41, 162
Gold, Tobias · 12, 30, 58, 111
Gonzalez, Laura · 38, 148
Goodall, Zachary · 24, 37, 96, 142
Goos, Carina · 15, 67
Górna, Maria · 15, 67
Gray, Sophie · 42, 168

H

Haenni, Beat · 9, 48
Hagedorn, Nicolas · 12, 30, 58, 111
Haindrich, Alexander · 41, 161
Hakim, Jill · 36, 139
Hampton, Marshall · 16, 32, 73, 121
Hamza, Iqbal · 40, 156
Harb, Omar S. · 41, 159
Harel, Amnon · 21, 87
Hartleb, Laura · 21, 88
Hashimoto, Hideharu · 21, 27, 86, 88, 104
Hauf, Laura · 30, 112
Heslop, Rhiannon · 13, 31, 62, 116
Hierro Yap, Carolina · 18, 40, 79, 155
Hodges, Kelly R · 9, 46
Hofer, Peter · 12, 54
Holder, Bailey · 19, 81
Holmes, Nikki A · 9, 46
Horáková, Eva · 15, 65
Horn, David · 10, 16, 21, 27, 31, 32, 50, 71, 72, 85, 104, 119, 120
Hossain, Zakir · 13, 31, 63, 117
Hovel-Miner, Galadriel · 13, 31, 61, 115
Hu, Jason · 24, 98
Huang, Lan · 24, 25, 98, 100
Husova, Michaela · 18, 79

I

Imhof, Axel · 37, 145
Isebe, Tony · 27, 42, 103, 170
Ishii, Midori · 15, 69
Ishikawa, Takashi · 9, 48
Ivens, Al · 24, 37, 96, 142

J

Jackson-Jones, Lucy · 19, 80
Jain, Minal · 40, 155
Jakimovska Ozdemir, Milena · 38, 147
Janzen, Christian J. · 12, 21, 30, 36, 58, 88, 111, 134
Ji, Zeyang · 27, 106
Juliano, John · 36, 139

K

Kachale, Ambar · 15, 65
Karolak, Natalia · 15, 67
Keneskhanova, Zhibek · 41, 157, 171
Kern, Mara · 9, 48
Kharabora, Oksana · 36, 139
Kim, Hee-Sook · 13, 31, 63, 117
Kirimlishvili, Sophio · 37, 41, 144, 164
Kiselar, Janna · 21, 88
Klebanov-Akopyan, Olga · 21, 87
Klingbeil, Michele M · 22, 90
Knight, Colin D · 35, 130
Kolev, Nikolay G · 41, 158
Kramer, Susanne · 15, 32, 37, 42, 67, 126, 141, 165
Krasilnikova, Marija · 28, 107
Krüger, Timothy · 9, 32, 45, 126
Krylova, Anna · 15, 67
Kunzová, Michaela · 18, 78
Kutova, Oksana · 27, 103

L

Lapsley, Craig · 22, 28, 89, 107
Larcombe, Stephen D · 12, 57
Lee, Jane · 24, 98
Leiss, Kayla · 37, 144
Lewis, Isaac · 24, 99
Li, Bibo · 27, 42, 106, 166
Li, Xiaorun · 24, 98
Li, Zihang · 24, 98
Lira, Andressa Brito · 40, 154
Lisack, Jaime N · 12, 56
Liu, Shiheng · 24, 98
Londoño, Paula A.C. · 15, 67
Loock, Mira · 13, 27, 31, 40, 62, 103, 116, 154
Lopez Escobar, Lara · 38, 147
Lopez, Jacqueline · 18, 36, 77, 140
Lukeš, Julius · 15, 65
Luzak, Vanessa · 15, 68

M

Macêdo, Juan P. · 41, 161
Machado, Henrique · 12, 54
Madmoni, Hava · 22, 40, 93, 153
Malaga Machaca, Edith · 36, 139
Maldonado, Rosa A. · 18, 35, 77, 130
Malenica, Kristina · 37, 145
Malfara, Madeline F · 9, 10, 30, 46, 52, 110
Maran, Suellen R · 10, 30, 51, 109
Marques, Catarina A · 16, 22, 27, 28, 31, 35, 40, 71, 91, 104, 107, 119, 133, 151
Matiz, Juan · 38, 148
Matthews, Keith R · 35
Matthews, Keith R · 12
Matthews, Keith R · 55
Matthews, Keith R · 57
Matthews, Keith R · 133
McAdams, Natialie M · 42, 169
McCall, Laura Isobel · 18, 77
McCulloch, Richard · 22, 28, 40, 89, 91, 107, 151
McDermott, Suzanne · 24, 36, 96, 99, 137, 142
McWilliam, Kirsty R · 40, 42, 157, 171
Meduiña, Maria Carmen · 36, 139
Meehan, Joshua · 24, 37, 96, 142
Mendes, Maria T · 18, 76, 77
Michael, Katja · 18, 35, 77, 130
Michaeli, Shulamit · 15, 22, 40, 66, 93, 153
Miletínová, Petra · 15, 65
Mirzavand Borujeni, Poorya · 16, 32, 73, 121
Mishra, Amartya · 24, 97
Monic, Sarah Gaëlle · 42, 167
Montoya, Alba · 35, 130
Moos, Martin · 18, 79
Moreira, Claudia · 15, 37, 67, 141
Moreira, Otacilio C. · 18, 36, 77, 140
Moreira-Leite, Flavia · 9, 47
Moretti, Nilmar S · 10, 30, 51, 109

Mottram, Jeremy C. · 16, 31, 41, 70, 118, 162
Mugnier, Monica R · 22, 27, 36, 40, 92, 102, 139, 146, 152
Mukherjee, Aditi · 13, 31, 63, 117
Murali, Shravya · 9, 50
Murrow, Jenna · 13, 31, 61, 115

N

Naguleswaran, Arunasalam · 37, 143
Neish, Rachel · 16, 31, 70, 118
Nelson, Scott W · 22, 90
Nenarokov, Serafim · 15, 65
Nenarokova, Anna · 15, 65
Newling, Katherine · 16, 31, 70, 118
Noga, Akira · 9, 48
Novotná, Markéta · 21, 85

O

Ochsenreiter, Torsten · 9, 48
Odenwald, Johanna · 15, 32, 37, 42, 67, 126, 141, 165
Oeljeklaus, Silke · 35, 132
Oldrieve, Guy · 12, 55
Oliveres, Quentin · 41, 161
Onn, Itay · 21, 87
Ortega-Rodriguez, Uriel · 18, 77
Otto, Thomas Dan · 35, 133
Ouarné, Marie · 12, 30, 59, 113

P

Pagura, Lucas · 18, 76
Pan, Xuehua · 27, 106
Panicucci, Brian · 18, 40, 79, 155
Papini Gabiatti, Bernardo · 32, 126
Paris, Zdeněk · 15, 65
Parman, Kristina · 19, 81
Parra-Muñoz, Marcela · 38, 148
Parry, Ewan · 16, 31, 70, 118
Paul, Kimberly · 19, 81
Pavlíková, Zuzana · 15, 65
Pereira, Alexa · 10, 30, 52, 110
Pérez-Antón, Elena · 13, 30, 60, 114
Perry, Melissa · 40, 156
Petersen, Austin · 32, 125
Piller, Silvan · 41, 161
Plevin, Michael J. · 16, 31, 70, 118
Portillo, Susana · 18, 35, 77, 130
Poudyal, Nava · 19, 81
Povelones, Megan L · 9, 10, 30, 46, 52, 110
Praisler, Carina · 12, 56
Pruzinova, Katerina · 9, 47
Pulido-Gomez, Solangie · 19, 81

R

Rabuffo, Claudia · 21, 84
Radecke, Julika · 9, 48
Rajan, K. Shanmugha · 15, 22, 40, 66, 93, 153
Rajesh, Rishi · 27, 103
Rancho-Jensen, Alisha · 37, 144
Raper, Jayne · 37, 41, 144, 164
Rauh, Elisa Theres · 12, 30, 35, 58, 111, 134
Rea, Edward · 9, 47
Read, Laurie K · 24, 37, 42, 97, 143, 169
Reichert, Andrea · 15, 67
Reis-Cunha, João · 22, 89
Rentsch, Doris · 41, 161
Rijo-Ferreira, Filipa · 27, 102
Rivera, Pilarita · 28, 107
Rodirguez, Maria P. · 38, 148
Roditi, Isabel · 15, 37, 66, 143
Rodriguez, Aura · 38, 148
Rodriguez, Felipe · 35, 130
Roithová, Adriana · 15, 65
Rojas Sánchez, Saúl · 41, 163
Roos, David S. · 41, 159
Rotureau, Brice · 13, 31, 60, 114
Rouskin, Silvi · 24, 96
Rudenko, Gloria · 27, 105
Rumbley, Jon · 32, 125

S

Sádlová, Jovana · 9, 47
Saha, Arpita · 27, 106
Salavati, Reza · 16, 32, 73, 121
Saliutama, Joshua · 19, 81
Sayeed, SK Abdus · 42, 166
Schenkman, Sergio · 10, 30, 51, 109
Schleicher, Ulrike · 12, 30, 36, 58, 111, 134
Schmidt, Markus · 21, 84
Schnaufer, Achim · 24, 37, 40, 96, 142, 155
Schneider, André · 35, 132
Schuster, Sarah · 12, 56
Serre, Karine · 12, 30, 59, 113
Sezerman, Ugur · 38, 147
Sharma, Sunil Kumar · 37, 142
Shen, Siqi · 32, 37, 42, 124, 141, 165
Shi, Huafang · 41, 158
Shlomai, Joseph · 21, 87
Siebert, Alistair · 9, 48
Siegel, T. Nicolai · 15, 21, 40, 68, 84, 157, 160, 171
Silva Pereira, Sara · 12, 30, 59, 113
Silva, Gabriel L A · 36, 138
Silva, Nadjania S de L · 10, 30, 51, 109
Simek, Petr · 18, 79
Singh, Vivek · 16, 32, 74, 122
Sladewski, Thomas E · 35, 131
Smith Jr., Joseph T. · 37, 143
Smith, Jaclyn E · 22, 37, 40, 92, 146, 152
Smoniewski, Clara · 16, 32, 73, 121
Soni, Awakash · 21, 87
Sortino, Katherine · 42, 169
Spangler, Dan · 19, 81
Stadler, Alexander · 9, 49
Starns, David · 41, 159
Sternlieb, Tamara · 13, 31, 40, 62, 116, 154
Stuart, Ken · 24, 36, 99, 137
Subrtova, Karolina · 40, 155
Sudlow, Sarah · 22, 40, 92, 152
Suematsu, Takuma · 25, 100
Sun, Yijun · 24, 42, 97, 169
Sunter, Jack · 9, 41, 47, 49, 162
Szoor, Balazs · 12, 57

T

Taleva, Gergana · 18, 79
Teles, Natalia M.M. · 16, 31, 70, 118
Thomson, Russell · 41, 164
Tiengwe, Calvin · 22, 40, 91, 151
Tinajeros, Freddy · 36, 139
Tinti, Michele · 9, 16, 27, 31, 50, 71, 72, 104, 119, 120
Torrecilhas, Ana C · 10, 30, 51, 109
Tosi, Luiz R O · 36, 138
Touray, Abdoulie O. · 27, 103
Tracy, Maxwell · 24, 99
Trenaman, Anna · 16, 31, 72, 120
Trindade, Sandra · 38, 147
Tschudi, Christian · 15, 41, 66, 158, 163
Tylec, Brianna · 37, 42, 143, 169

U

Unger, Ron · 15, 22, 40, 66, 93, 153
Urbaniak, Michael · 19, 33, 80, 127

Urrea, Daniel · 38, 148

V

Vacas, Andres · 25, 100
Valášek, Leoš Shivaya · 15, 65
Van den Broeck, Frederik · 12, 55
VandeBerg, Jane · 18, 77
VandeBerg, John L. · 18, 77
Vargas, Carolina · 38, 148
Vaughan, Sue · 9, 49
Verastegui, Manuela · 36, 139
Vinales, Irodiel · 35, 130
Volbeer, Brianna · 19, 81
Volf, Petr · 9, 47
von Känel, Corinne · 35, 132
Votýpka, Jan · 15, 65

W

Wall, Richard · 16, 32, 72, 120
Walrad, Pegine B. · 16, 31, 70, 118
Waltmann, Andreea · 36, 139
Wang, Hong · 24, 98
Wang, Kam-Bo · 27, 106
Wang, Kevin J · 37, 146
Warscheid, Bettina · 35, 132
Wheeler, Richard J · 36, 41, 135, 160, 162, 168
Won, Madalyn M · 9, 38, 45, 149
Wong, Jonathan · 16, 32, 74, 122
Woo, Jia · 24, 96
Working Group on Chagas Disease in Bolivia and Peru · 36, 139
Worthington, John · 19, 80

Y

Yadav, Prateek · 41, 160
Yamin, Katreena · 21, 87
Yanase, Ryuji · 9, 47
Yang, Xian · 27, 106
Yates, Phil · 40, 156
Yu, Clinton · 24, 25, 98, 100
Yu, Zihao · 24, 96
Yurchenko, Vyacheslav · 15, 65

Z

Záhonová, Kristína · 15, 65
Zavřelová, Anna · 32, 124
Zechner, Rudolf · 12, 54
Zeng, Lanying · 24, 96
Zhang, Bailin · 27, 102
Zhang, Fan · 24, 98
Zhang, Liye · 24, 98
Zhang, Mingjie · 27, 106
Zhang, Xiren · 24, 96
Zhang, Xiuren · 37, 142
Zhao, Yanxiang · 27, 106
Zhou, Z. Hong · 24, 98
Zíková, Alena · 16, 18, 32, 40, 74, 78, 79, 122, 155
Zimmer, Sara L · 16, 19, 32, 36, 73, 82, 121, 125, 136
Zoltner, Martin · 15, 32, 37, 42, 67, 124, 141, 165
Zuber, Benoît · 9, 48