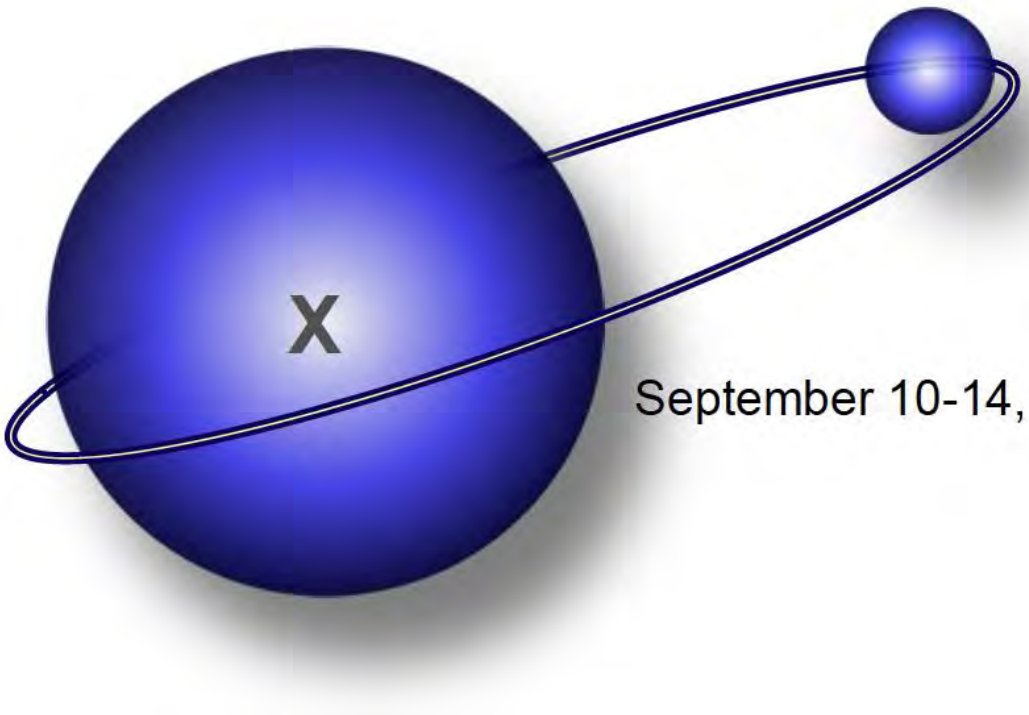
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory. Woods Hole, MA. USA



September 10-14, 2024

Tenth Kinetoplastid Molecular Cell Biology Meeting
KMCBM X

September 10th – 14th, 2024

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

Origins of KMCBM

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

Year	Organizers
2005	George A.M. Cross
2007	George A.M. Cross
2009	George A.M. Cross
2011	George A.M. Cross
2013	Christian Tschudi
2015	Christian Tschudi
2017	Christian Tschudi
2019	Christian Tschudi
2022	Christian Tschudi & Luisa M. Figueiredo
2024	Luisa M. Figueiredo, Chris de Graffenried, Megan L. Povelones & Omar S. Harb

Alice and C. C. Wang award



David S. Roos – 2024 Award recipient



David A. Fidock – 2025 Award recipient

Congratulations to David S. Roos (University of Pennsylvania) and David A. Fidock (Columbia University) for receiving the 2024 and 2025 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: Professor Gloria Rudenko 1961-2022

Professor Gloria Rudenko who studied trypanosome parasites that cause African sleeping sickness sadly passed away on November 24th, 2022, following a long, heroic battle with cancer.



She is best known for studying how trypanosomes switch their surface coat as a strategy to escape from antibody attack during a chronic infection. This coat is formed from a single protein known as the Variant Surface Glycoprotein (VSG) and protects the trypanosome from host-derived antibodies. The process of continuous switching of the VSG surface coat is referred to as antigenic variation.

Gloria made seminal discoveries regarding our understanding of how production of the VSG surface coat is connected to cell cycle progression in African trypanosomes and how different splicing factories facilitate production of high levels of VSG mRNAs. Her early work discovered that trypanosomes use RNA polymerase I (Pol I) to express procyclin, the major surface protein of insect stage parasites. This is unusual as other eukaryotes only use Pol I to transcribe rDNA, and no other eukaryote has been shown till date to use Pol I to transcribe protein coding genes.

Gloria touched so many lives and was an exceedingly caring, popular member of staff who was a gifted, inspirational scientist and teacher. She was a dedicated mentor to students and scientists at all levels, and a strong advocate for women scientists and ethnic minorities. She had an unrivalled dedication to her research. Despite her long-fought battle with cancer, she maintained her research program for an extended period. This is testimony to her persistence, resilience, and her love for science. She had an uncanny knack of what constitutes rigorous science, and this is evident in the quality of her work. She was a truly exceptional scientist.

Gloria joined the Department of Life Sciences at Imperial in 2010, as a Reader in Molecular Microbiology before being promoted to Professor of Molecular Microbiology in 2015. Before Imperial, she was a Wellcome Senior Fellow at the University of Oxford, and a postdoctoral fellow in the laboratory of Prof. Piet Borst at the Netherlands Cancer Institute, Amsterdam. She earned her PhD from the University of Amsterdam based on research performed in the Department of Genetics, Columbia University, New York, NY.
She will be sadly missed by all those who had the privilege and good fortune to know her, and especially by her students, research team and colleagues.

Contributed by Calvin Tiengwe (Imperial College London)

https://www.imperial.ac.uk/news/241991/celebrating-life-professor-gloria-rudenko-1961-2022/

In Memoriam: Professor Dan Ray 1937-2024

"There are a few people whose paths cross with yours that you recognize as truly extraordinary…… Dan Ray was one of those people."



Dr. Dan S. Ray, Professor Emeritus and UCLA scientist, passed away January 31, 2024. Dan has a deep legacy as a scientist, educator and a wonderful soul who has left long-lasting impressions with his many trainees and colleagues. Dan's 47-year career focused on the study of DNA replication. Using a virus called M13 as his model, he published a series of seven landmark papers in 1969 - 1971 that cemented Dan's reputation for excellence in the field as an Assistant Professor. In the 1980's Dan took on a new challenge, pioneering the study of DNA replication in kinetoplastid parasites, particularly of the mitochondrial DNA called kDNA. Dan was a brilliant and creative scientist whose excitement and enthusiasm for research never diminished.

Dan's educational efforts included mentorship and leadership, where as an Assistant Professor of Molecular Biology, he helped establish the Molecular Biology Interdepartmental Doctoral Program (MBIDP) in 1967. Today this program is still a vibrant interdisciplinary graduate program committed to training PhD candidates to become the next generation of scientific leaders.

At the time of his retirement in 2014, Dan had mentored upwards of 40 graduate students and postdoctoral fellows and several undergraduates. One notable undergraduate trainee is Randy Schekman (2013 Nobel Prize in Physiology or Medicine).

Dan's influence and impact on molecular parasitology was great, not only because of his expertise and passion for science, but most importantly because of his warmth, patience, enthusiasm, and supportive mentoring style. The teamwork of Dan and Jane Hines were essential to the insights that the Ray lab produced. Dan was quick to smile and always took great joy in discovery. His willingness to mentor new scientists into the field of molecular parasitology was notable, and new faculty members especially appreciated his sage advice.

Dan encouraged curiosity, and celebrated successes, and he will be missed greatly.

Contributed by Dr. Michele M Klingbeil, (University of Massachusetts)

https://www.legacy.com/us/obituaries/latimes/name/dan-ray-obituary?id=54345276

Acknowledgements

The organizers wish to thank:

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 2024 Annual Meeting, to be held November 13 – 17, 2024 in New Orleans, Louisiana.

The Staff at MBL:

Kim Elber 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

Sponsors:

Program

Tuesday September 10th 2024		
2:00PM – 5:00PM	Arrival and registration	Swope
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:00PM	Session I: Parasites: sensing and responding	Clapp
9:00PM – 11:00PM	Mixer	Swope
Wednesday September 11th 2024		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 11:30AM	Session II: Pushing methodology forward	Clapp
12:00PM – 1:30PM	Lunch	Swope
2:00PM – 4:45PM	Session III: Cell biology	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 12th 2024		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 11:30AM	Session IV: Genomes and their architecture	Clapp
12:00PM – 1:30PM	Lunch	Swope
1:45PM – 2:15PM	DISCUSSION: Present and Future of Pathogen Genome Resources	Clapp
2:15PM - 5:00 PM	Free time and ad hoc sessions	
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 13th 2024		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 11:30AM	Session V: The host perspective	Clapp
12:00PM – 1:30PM	Lunch	Swope
2:00PM – 4:45PM	Session VI: Insights from structures	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 14th 2024		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 11:00AM	Session VII: Gene regulation	Clapp
12:00PM – 1:30PM	Lunch	Swope

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

Standard Talk: 12 min; Turbo Talk (TT): 3 min; Plenary talk: 35 min

Session I

Parasites: sensing and responding

Chair: Calvin Tiengwe

Tuesday, September 10

7:00PM – 9:00PM

1 Single-cell transcriptomic heterogeneity of *Leishmania major* transmission and development in their natural sand fly vector

Carolina M.C. Catta-Preta (NIAID, NIH); Kashinath Ghosh (NIAID, NIH); David L. Sacks (NIAID, NIH); Tiago R. Ferreira (NIAID, NIH)

2 A single cell RNA sequencing atlas of *Trypanosoma cruzi* development reveals high resolution heterogeneity in the parasite lifecycle

Ross F Laidlaw (University of Glasgow); Marta Garcia-Sanchez (University of Dundee); Juliana Da Silva Pacheco (University of Dundee); Luciana De Sousa Paradela (University of Dundee); Thomas D Otto (University of Glasgow); Manu De Rycker (University of Dundee)

3 Hide and Go Seq: Capturing the Antibody-VSG Arms Race During *Trypanosoma brucei* Infection

Lulu M Singer (Johns Hopkins Bloomberg School of Public Health); Jaime E So (Johns Hopkins University School of Medicine); Alexander K Beaver (Johns Hopkins University School of Medicine); Monica R Mugnier (Johns Hopkins Bloomberg School of Public Health)

4 Nutritional stress as a signal for *Trypanosoma brucei* differentiation to the mesocyclic-like form

Eva Dolezelova (Biology Centre, CAS); Hana Pavliskova (Institute of Molecular Genetics, CAS); Tomas Skalicky (Biology Centre, CAS); Martin Moos (Biology Centre, CAS); Vladimir Varga (Institute of Molecular Genetics, CAS); Alena Zikova (Biology Centre, CAS)

5 Proteomic and lipidomic profiles reveal phospholipase-dependent adaptation to heat-stress in African trypanosomes

Gustavo Bravo Ruiz (University of Dundee); Rupa Nagar (University of Dundee); Michele Tinti (University of Dundee); Michael Ferguson (University of Dundee); David Horn (University of Dundee)

6 *Leishmania* ESB1 ortholog regulates delta amastin expression

Jorge Arias del Angel (Oxford Brookes University); Richard J Wheeler (University of Edinburgh); Jack D Sunter (Oxford Brookes University)

7 Genome-wide fitness profiling by DRiF-Seq identifies novel regulators acting early in the *Trypanosoma brucei* quorum sensing signalling pathway

Kirsty R McWilliam (The University of Edinburgh); Simon D'Archivio (The University of Nottingham); Phoebe Kim (The University of Edinburgh); Olga Dluzniewska (The University of Edinburgh); Catarina Gadelha (The University of Nottingham); Keith R Matthews (The University of Edinburgh)

8 (TT) Decoding single cell gene expression as parasites invade the adipose tissue

Lara López-Escobar (Instituto de Medicina Molecular); Milena Jakimovska Özdemir (Acibadem University); Ugur Sezerman (Acibadem University); Luisa M Figueiredo (Instituto de Medicina Molecular)

Session II

Pushing methodology forward

Chair: Joana Faria

Wednesday, September 11

8:45AM – 11:30AM

9 (Plenary) The TrypanoGEN genome-wide association study of susceptibility to trypanosomiasis and insights in the evolutionary trade-off between resistance to infection and susceptivity to non-communicable diseases

Annette MacLeod, for the TrypanoGEN Research Group, members of The H3Africa Consortium: Julius Mulindwa, Magambo Phillip Kimuda, Harry Noyes, Hamidou Ilboudo, Mathurin Koffi, Bernadin Ahouty, Oscar Nyangiri, Anneli Cooper, Caroline Clucas, Peter Nambala, Walt Adamson, Janelisa Musaya, Dieudonne´ Mumba Ngoyi, Kevin Karume, Olivier Fataki, Gustave Simo, Elvis Ofon, John Enyaru, Barbara Nerima, Andy Tait, Lucio Marcello, John Chisi, Jacques Kabore, Justin Windingoudi Kabore, Kelita Kamoto, Martin Simuunza,Vincent P. Alibu, Vincent Jamonneau, Marianne Camera, Mamadou Camara, Bruno Bucheton, Christiane Hertz-Fowler and Enock Matovu

10 New insights into the biology of dermal trypanosomes using a skin-on-chip organoid model

Parul Sharma (Institut Pasteur); Christelle Travaillé (Institut Pasteur); Samy Gobaa (Institut Pasteur); Brice Rotureau (Institut Pasteur)

11 Growth and transcriptomics of micropopulations in semi-permeable capsules to quantify VSG switching frequencies

Anna Barcons-Simon (LMU Munich); Kiryl Kavaliou (LMU Munich); Esteban Osses Soto (LMU Munich); T. Nicolai Siegel (LMU Munich)

12 Molecular tools for functional genetics in *Trypanosoma* vivax

Majeed Bakari-Soale (University of Nottingham); Pieter C Steketee (The Roslin Institute); Rachel Young (The Roslin Institute); Federica Giordani (University of Glasgow); Michael P Barrett (University of Glasgow); Liam Morrison (The Roslin Institute); Bill Wickstead (University of Nottingham); Catarina Gadelha (University of Nottingham)

COFFEE BREAK

13 Establishment of a new system to study in vivo interactions of *Trypanosoma cruzi* and its triatomine insect vector

Ruby E Harrison (University of Georgia CTEGD and Dept of Cellular Biology); Kevin J Vogel (University of Georgia Dept of Entomology); R Drew Etheridge (University of Georgia CTEGD and Dept of Cellular Biology)

14 (TT) Identifying molecular mediators of cellular states in *Trypanosoma cruzi*: comparison of single cell RNA sequencing technologies

Caroline D Keroack (Brown University); Sophie Marcus-Wade (Brown University); Jacqueline Lopez (Brown University); Eric D Salomaki (Brown University); Christopher L de Graffenried (Brown University)

15 (TT) LeishGEM: A genome-scale database for knockout mutant life cycle fitness phenotyping and subcellular protein localisation in *Leishmania mexicana*

Ulrich Dobramysl (University of Oxford); Eden R Ferreira (University of York); Rachel P Neish (University of York); Laura D Davidson (Oxford Brookes University); Raquel Pereira (University of Bern); Ruth Etzensperger (University of Bern); Sidonie Aellig (University of Bern); Matthew Young (University of Glasgow); James Smith (University of Glasgow); Jeziel Damasceno (University of Glasgow); Jack D Sunter (Oxford Brookes University); Jeremy Mottram (University of York); Eva Gluenz (University of Bern); Richard Wheeler (University of Oxford)

16 (TT) A novel conditional knockdown system in *T. cruzi* reveals essential roles for Aurora and Polo-like kinases in parasite cell division

Justin Wiedeman (University of Georgia); Ruby Harrison (University of Georgia); Drew Etheridge (University of Georgia)

17 (TT) A 2A peptide-based epitope-tagging toolkit for analysis of protein localisation, regulation, and function in Trypanosomatids

Carla Gilabert Carbajo (Imperial College London); Xiaoyang Han (Imperial College London); Bhairavi Savur (Imperial College London); Arushi Upadhyaya (Imperial College London); Fatima Taha (Imperial College London); Richard J Wheeler (University of Oxford); Michele Tinti (University of Dundee); Phillip Yates (Oregon Health and Science University); Calvin Tiengwe (Imperial College London)

Session III

Cell biology

Chair: Joseph Smith

Wednesday, September 11

2:00PM – 4:45PM

18 *Leishmania mexicana* flagellum attachment zone is required for adhesion to substrates

Barrack O Owino (Oxford Brookes University); Ryuji Yanase (University of Leicester); Alan Marron (Oxford Brookes University); Flavia Moreira-Leite (Oxford Brookes University); Sue Vaughan (Oxford Brookes University); Jack D Sunter (Oxford Brookes University)

19 A novel approach to tagging tubulin reveals microtubule assembly dynamics of the flagellum in *Trypanosoma brucei*

Daniel Abbühl (Institut Pasteur Paris); Serge Bonnefoy (Institut Pasteur Paris); Bastin Philippe (Institut Pasteur Paris)

20 Flagellar distal-end proteome is critical for axonemal construction and length regulation in *Trypanosoma brucei*

Hana Pavliskova (Institute of Molecular Genetics of the Czech Academy of Sciences); Peter Gorilak (Institute of Molecular Genetics of the Czech Academy of Sciences); Ludek Stepanek (Institute of Molecular Genetics of the Czech Academy of Sciences); Marie Zelena (Institute of Molecular Genetics of the Czech Academy of Sciences); Bill Wickstead (University of Nottingham); Vladimir Varga (Institute of Molecular Genetics of the Czech Academy of Sciences)

21 Tubulin detyrosination shapes *Leishmania* cytoskeletal architecture and virulence

Rosa Milagros Corrales (University of Montpellier, CNRS, IRD, MiVEGEC); Jeremy Vincent (University of Montpellier, CNRS, IRD, MiVEGEC); Lucien Crobu (University of Montpellier, CNRS, IRD, MiVEGEC); Rachel Neish (York Biomedical Research Institute and Department of Biology, University of York); Binita Nepal (Department of Pediatrics and Department of Biochemistry, University of Texas Southwestern Medical Center); Julien Espeut (Tubulin Code team, IGH, CNRS); Grégoire Pasquier (University of Montpellier, CNRS, IRD, MiVEGEC); Ghislain Gillard (Tubulin Code team, IGH, CNRS); Chantal Cazevieille (Université Montpellier, INSERM U1298, INM); Jeremy Mottram (York Biomedical Research Institute and Department of Biology, University of York); Dawn Wetzel (Department of Pediatrics and Department of Biochemistry, University of Texas Southwestern Medical Center); Yvon Sterkers (University of Montpellier, CNRS, IRD, MiVEGEC); Krzysztof Rogowski (Tubulin Code team, IGH, CNRS); Maude F Leveque (University of Montpellier, CNRS, IRD, MiVEGEC)

22 How to attach the TAC

Salome Aeschlimann (DCBP, University of Bern); Caroline Dewar (University of Lancaster); Clirim Jetishi (ICB, University of Bern); Bianca Berger (ICB, University of Bern); Bernd Schimanski (DCBP, University of Bern); Philip Stettler (DCBP, University of Bern); Silke Oljeklaus (Biozentrum, University of Würzburg); Bettina Warscheid (Biozentrum, University of Würzburg); Torsten Ochsenreiter (ICB, University of Bern); André Schneider (DCBP, University of Bern)

COFFEE BREAK

23 Better together: the importance of ATP synthase dimerization for cristae maturation and OXPHOS

Michaela Husová (Biology Centre, Institute of Parasitology; The University of South Bohemia, Faculty of Science); Martin Zoltner (School of Life Sciences, University of Dundee; Department of Parasitology, Faculty of Science, BIOCEV, Charles University); Mark Field (Biology Centre, Institute of Parasitology; School of Life Sciences, University of Dundee); Alena Zíková (Biology Centre, Institute of Parasitology; The University of South Bohemia, Faculty of Science)

24 Functional characterization of compartmentalized cAMP signals in *Trypanosoma cruzi*

Milad Ahmed (University of Cincinnati); Miguel Chiurillo (University of Cincinnati); Noelia Lander (University of Cincinnati)

25 (TT) The hydrophobic effect drives the outer-to-inner mitochondrial membrane connection of the Trypanosomal tripartite attachment complex

Philip Stettler (University of Bern); Salome Aeschlimann (University of Bern); Bernd Schimanski (University of Bern); Sandro Käser (University of Bern); André Schneider (University of Bern)

26 (TT) Bicarbonate – CO_2 sensing and response mediated by trypanosome QIQ1

Anna Trenaman (University of Dundee); Federico Rojas (University of Edinburgh); Michele Tinti (University of Dundee); Keith Matthews (University of Edinburgh); Sam Alsford (London School of Hygiene and Tropical Medicine); David Horn (University of Dundee)

27 (TT) A genome-wide overexpression screen identifies proteins that inhibits the growth of *Trypanosoma brucei*

Navina Panneer Selvan (Rutgers University); Aditi Mukherjee (Rutgers University); Esteban Erben (IIBIO-UNSAM); Hee-Sook Kim (Rutgers University)

28 (TT) Host-pathogen dynamics of bumble bee parasite, *Crithidia bombi*

Blyssalyn V Bieber (Villanova University); Faith St. Clair (Villanova University); Sarah Lockett (Villanova University); Sonja Glasser (University of Massachusetts Amherst); Lynn S Adler (University of Massachusetts Amherst); Megan L Povelones (Villanova University)

Session IV

Genomes and their architecture

Chair: Gaelle Lentini

Thursday, September 12

8:45AM – 11:30AM

29 (Plenary) Mechanisms of antigen diversification in *Trypanosoma brucei*

Monica Mugnier (Johns Hopkins School of Public Health)

30 Nanopore sequencing-based deep learning assay reveals the complete DNA replication landscape in *Leishmania* and its connection with genome variability

Jeziel D. Damasceno (University of Glasgow); Gabriel L.A. da Silva (University of Glasgow); Catarina A. Marques (University of Glasgow); Craig Lapsley (University of Glasgow); Dario Beraldi (University of Glasgow); Richard McCulloch (University of Glasgow)

31 Structural characterisation of nucleosomes from *Trypanosoma brucei* reveals unique features of the Trypanosomatid chromatin landscape

Gauri Deák (University of Edinburgh); Hannah Wapenaar (University of Edinburgh); Gorka Sandoval (University of Edinburgh); Hayden Burdett (University of Edinburgh); Ruofan Chen (University of Edinburgh); Mark Taylor (University of Edinburgh); James Watson (University of Edinburgh); Maarten Tuijtel (University of Edinburgh); Martin Singleton (University of Edinburgh); Shaun Webb (University of Edinburgh); Marcus Wilson (University of Edinburgh)

32 Comparative analysis of canonical and non-canonical nuclear genetic codes in Trypanosomatids

Kristina Pasutova (Institute of Parasitology); Zoltan Fussy (Scripps Institution of Oceanography); Amanda Albanaz (University of Ostrava); Jan Votypka (Institute of Prasitology); Anzhelika Butenko (Institute of Parasitology); Ambar Kachale (Institute of Parasitology); Alexei Kostigov (University of Ostrava); Vyacheslav Yurchenko (University of Ostrava); Julius Lukes (Institute of Parasitology)

COFFEE BREAK

33 Chromatin three-dimensional organization into compartments and domains in *Trypanosoma cruzi*

Florencia Díaz-Viraqué (Institut Pasteur Montevideo); María L Chiribao (Institut Pasteur de Montevideo; UdelaR); Gabriela Libisch (Institut Pasteur Montevideo); Carlos Robello (Institut Pasteur de Montevideo; UdelaR)

34 Hidden origami in *Trypanosoma cruzi* nuclei highlights its nonrandom 3D genomic organization

Natália Karla Bellini (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of

Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil); Pedro Leonardo Carvalho de Lima (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil); David da Silva Pires (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil); Julia Pinheiro Chagas da Cunha (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil)

35 (TT) High-accuracy genome assembly reveals hotspots for large gene families in *Trypanosoma cruzi* chromosomes

Lissa Cruz-Saavedra (McGill University); Luiza Berenguer Antunes (McGill University); Mira Loock (McGill University); Igor Cestari (McGill University)

36 (TT) Cross-linking and mass spectrometry uncovers chromatin compartment proteins and subtelomeric VSG gene silencing

Tony Isebe (McGill University); Luiza Antunes (McGill University); Oksana Kutova (McGill University); Igor Cestari (McGill University)

DISCUSSION

Present and future of pathogen genome resources

Chairs: Luisa M Figueiredo, Chris de Graffenried and Megan Povelones

Thursday, September 12

1:45PM – 2:15PM

37 TriTrypDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration, Discovery, and Dissemination

David S. Roos (Univ Pennsylvania… on behalf of the VEuPathDB Bioinformatics Resource Center)

38 The NIAID Bioinformatics Resources Centers (BRCs) for Infectious Diseases: accelerating bioinformatics for parasites, bacterial, fungal, vector, and viral infectious disease research

Liliana Brown (NIAID); Wiriya Rutvisuttinunt (NIAID)

Ad hoc sessions:

Running a research program with undergraduates in a teaching focused faculty position: presentations and panel discussion
Chair: Danae Schulz (Harvey Mudd College)

Session V

The host perspective

Chair: Federico Rojas

Friday, September 13

8:45AM – 11:30AM

39 (Plenary) Time to consider *Trypanosoma cruzi*

Rick L. Tarleton (Center for Tropical and Emerging Global Diseases, University of Georgia)

40 Identifying vaccine targets for Chagas disease using yeast surface display screens

Mira Loock (McGill University); Valeria B Araujo (McGill University); Luiza B Antunes (McGill University); Igor Cestari (McGill University)

41 Fexinidazole induced cytotoxicity is distinct from related anti-trypanosome nitroaromatic drugs

Kenna Berg (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Indea Rogers (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Hayley Ramirez (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Ignacio Durante (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Julian Cornejo (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Paprika Berry (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Galadriel Hovel-Miner (Stony Brook University, Renaissance School of Medicine, Department of Microbiology and Immunology)

42 Resistance-associated mutations in the target of acoziborole - trypanosome cleavage and polyadenylation specificity factor 3

Melanie C Ridgway (University of Dundee); Michele Tinti (University of Dundee); David Horn (University of Dundee)

COFFEE BREAK

43 The local immune response to *Trypanosoma brucei* in the tissues of the abdomen

Chloe Barnes (Lancaster University); Megan Dooley (Lancaster University); Nadia Iqbal (Lancaster University); Alex Hardgrave (Lancaster University); Sheila Macharia (Lancaster University); Ivo Basilio-Queijo (Lancaster University); Otto Wheeler (Lancaster University); Neil Dawson (Lancaster University); Lucy Jackson-Jones (Lancaster University); John Worthington (Lancaster University); Mick Urbaniak (Lancaster University)

44 Understanding weight loss in mice infected by *Trypanosoma brucei*

David Ferreira (Instituto de Medicina Molecular); Henrique Machado (Instituto de Medicina Molecular); Sandra Trindade (Instituto de Medicina Molecular); Abdulbasit Amin (Instituto de Medicina Molecular); Luisa M Figueiredo (Instituto de Medicina Molecular)

45 (TT) Genome analysis of *T. cruzi* field isolates offers the opportunity to study the effect of infection context on parasite genetic diversity

Jill Hakim (Johns Hopkins); Sneider Gutiérrez (Johns Hopkins); Edith Malaga (Universidad Cayetano Herida); Louisa Messenger (University of Arizona); Monica Mugnier (Johns Hopkins)

46 (TT) Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models

Sara Fresard (CUNY Hunter College, The Graduate Center at City University of New York); Kayla Leiss (CUNY Hunter College); Russell Thomson (CUNY Hunter College); Jayne Raper (CUNY Hunter College, The Graduate Center at City University of New York)

47 (TT) Host response to cutaneous Human African Trypanosomiasis

Rhiannon Heslop (University of Glasgow); Nono-Raymond Kuispond Swar (National Institute for Biomedical Research, Kinshasa); Anneli Cooper (University of Glasgow); Paul Capewell (University of Glasgow); Dieudonne Mumba Ngoyi (National Institute for Biomedical Research, Kinshasa); Annette MacLeod (University of Glasgow)

Session VI

Insights from structures

Chair: Stella Sun

Friday, September 13

2:00PM – 4:45PM

48 Structural and functional insights into ESAG3, a putative glycosyltransferase, in African trypanosomes

Qi Zhong (Imperial College London); Joseph D Barritt (Imperial College London); Carla Gilabert Carbajo (Imperial College London); Emmanuel Nji (Centre for Research in Infectious Diseases); Samuel Dean (University of Warwick); Michele Tinti (University of Dundee); Michael A.J. Ferguson (University of Dundee); Erhard Hohenester (Imperial College London); Sarah Rouse (Imperial College London); Calvin Tiengwe (Imperial College London)

49 Structural studies of CFAP410/C21orf2 reveal a bimodular organization with two distinct domains for its localization and function

Alexander Stadler (Medical University of Vienna); Laryssa V. De Liz (Oxford Brookes University); Heloisa Gabriel (Oxford Brookes University); Santiago Alonso-Gil (University of Vienna); Robbie Crickley (Oxford Brookes University); Katharina Korbula (Medical University of Vienna); Bojan Zagrovic (University of Vienna); Sue Vaughan (Oxford Brookes University); Jack D. Sunter (Oxford Brookes University); Gang Dong (Medical University of Vienna)

50 Structures of mitochondrial RNA editing catalytic complexes from *T. brucei*

Ruslan Afasizhev (Boston University); Yun-Tao Liu (UCLA); Andres Vacas (Boston University); Jane Lee (UCLA); Xiaojing Zhao (Shanghai Tech); Jonathan Jih (UCLA); Takuma Suematsu (Boston University); Clinton Yu (UC Irvine); Lan Huang (UC Irvine); Liye Zhang (Shanghai Tech); Inna Afasizheva (Boston University); Hong Zhou (UCLA)

51 Mechanistic Insights into KREH2C-Mediated gRNA Displacement in Trypanosomal RNA Editing

Shiheng Liu (University of California, Los Angeles); Takuma Suematsu (Boston University Medical Campus); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine); Liye Zhang (ShanghaiTech University); Inna Afasizheva (Boston University Medical Campus); Z. Hong Zhou (University of California, Los Angeles); Ruslan Afasizhev (Boston University Medical Campus)

52 Domain structure and function in RNA Editing Catalytic Complexes in *Trypanosoma brucei*

Jason Carnes (Seattle Children's Research Institute); Suzanne M. McDermott (Seattle Children's Research Institute); Brittney Davidge (Seattle Children's Research Institute); Tyler Rodshagen (Seattle Children's Research Institute); Isaac Lewis (Seattle Children's Research Institute); Maxwell Tracy (Seattle Children's Research Institute); Kenneth Stuart (Seattle Children's Research Institute)

COFFEE BREAK

53 The small nucleolar RNAs are master regulators controlling ribosome structure and function and mRNA fate in trypanosomes and *Leishmania*

K Shanmugha Rajan (Weizmann Institute); Saurav Aryal (Bar-Ilan University Beathrice); Beathrice Galili Kostin (Bar-Ilan University); Anat Bashan (Weizmann Institute); Mika Olami (Weizmann Institute); Hava Madmoni (Bar-Ilan University); Sharanya Murugeshan (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Ron Unger (Bar-Ilan University); Christian Tschudi (Yale university); Gerald F . Späth (Pasteur Institute); Ada Yonath (Weizmann Institute); Shulamit Michaeli (Bar-Ilan University)

54 The Neddylation pathway is a crucial regulator of *Trypanosoma brucei* differentiation

Federico Rojas (Lancaster University)

55 (TT) RNA viruses in *Blastocrithidia*: does a unique genetic code protect from viral infection?

Danyil Grybchuk (University of Ostrava); Donnamae Klocek (University of Ostrava); Alexei Kostygov (University of Ostrava); Vyacheslav Yurchenko (University of Ostrava)

56 (TT) REH2C Complex is the First Identified Regulatory Factor in Lifecycle Stage-specific RNA Editing Repression During *Trypanosoma brucei* Development

Cody Goode (Texas A&M University); Binny Kaur (Texas A&M University); Sunil Sharma (Texas A&M University); Zihao Chen (University of Edinburgh); Tyler Rodshagen (Seattle Children's Research Institute); Scott Grote (Harvard Medical School); Joshua Meehan (Texas A&M University); Al Ivens (University of Edinburgh); Nick J. Savill (University of Edinburgh); Silvi Rouskin (Harvard Medical School); Achim Schnaufer (University of Edinburgh); Suzanne M. McDermott (Seattle Children's Research Institute); Jorge Cruz-Reyes (Texas A&M University)

57 (TT) Deep mutational resistance profiling for anti-Trypanosomal proteasome inhibitors

Simone Altmann (University of Dundee); Michele Tinti (University of Dundee); Melanie Ridgway (University of Dundee); Manu de Rycker (University of Dundee); Michael Thomas (University of Dundee); Cesar Mendoza Martinez (University of Dundee); Jagmohan Saini (University of Dundee); Peter Ibrahim (University of Dundee); Mike Bodkin (University of Dundee); David Horn (University of Dundee)

58 (TT) Forging the One Ring of *Trypanosoma brucei*: Structural characterization of BILBO1 assembly

Kim Ivan Abesamis (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter); Arda Kara (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter); Derrick Robinson (Microbiologie Fondamentale et Pathogénicité, CNRS UMR5234, University of Bordeaux); Mélanie Bonhivers (Microbiologie Fondamentale et Pathogénicité, CNRS UMR5234, University of Bordeaux); Gang Dong (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter)

Session VII

Gene regulation

Chair: Danae Schulz

Saturday, September 14

8:45AM – 11:00AM

59 Challenging trypanosome antigenic variation paradigms using natural systems

Stephen D Larcombe (University of Edinburgh); Jane C Munday (University of Glasgow); Guy R Oldrieve (University of Edinburgh); COBALT Consortium

60 Novel expression-site body specific components finetune expression at the active-VSG expression-site in African trypanosomes

Lianne Lansink (University of York); Adam Dowle (University of York); Leon Walther (University of York); Joana Faria (University of York)

61 Repair template availability determines the VSG switching mechanism after a double-strand break in the active VSG in *Trypanosoma brucei*

Raúl O Cosentino (LMU Munich); Kirsty R McWilliam (LMU Munich); Zhibek Keneshkanova (LMU Munich); Atai Dobrynin (LMU Munich); Jaclyn E Smith (Johns Hopkins Bloomberg School of Public Health); Ines Subota (LMU Munich); Monica R Mugnier (Johns Hopkins Bloomberg School of Public Health); Maria Colomé-Tatché (LMU Munich); T. Nicolai Siegel (LMU Munich)

62 Identification of a novel protein that suppresses VSG switching in *Trypanosoma brucei*

Navina Panneer Selvan (PHRI, Rutgers University); Aditi Mukherjee (PHRI, Rutgers University); Eliezer Cruz (IIBIO, UNSAM, San Martín, Argentina); Vanina Campo (IIBIO, UNSAM, San Martín, Argentina); Esteban Erben (IIBIO, UNSAM, San Martín, Argentina); Hee-Sook Kim (PHRI, DMMG, Rutgers University)

63 Rab2B promotes chaperonin-mediated actin folding and prevents developmental transcription reprogramming and quiescence in trypanosomes

Feng-Jun Li (National University of Singapore); Cynthia Y. He (National University of Singapore)

64 The enigma of PP-IPs in the *Trypanosoma cruzi* life cycle

Thaise Lara Teixeira (University of São Paulo); Bryan Abuchery (University of São Paulo); Vitor Luiz da Silva (São Paulo State University); Bruno Alves Santarossa (Butantan Institute); Maria Cristina Motta (Federal University of Rio de Janeiro); Samuel Cota Teixeira (Federal University of Uberlândia); Eloisa Amalia Ferro (Federal University of Uberlândia); Simone Calderano (Butantan Institute); Miguel Angel Chiurrilo (University of Cincinnati); Noelia Lander (University of Cincinnati); Roberto Docampo (University of Georgia); Marcelo Santos da Silva (University of São Paulo)

65 Key *Leishmania* trans-regulators are essential for parasite surveillance and infectivity

Ewan Parry (University of York); Natalia M.M. Teles (University of York); Rachel Neish

(University of York); Adam Dowle (University of York); Katherine Newling (University of York); Eva Kyriakou (University of York); Fabiano Pais (University of York); Jeremy C. Mottram (University of York); Pegine B. Walrad (University of York)

Poster Session A

Wednesday, September 11

7:00PM

8 (TT) Decoding single cell gene expression as parasites invade the adipose tissue

Lara López-Escobar (Instituto de Medicina Molecular); Milena Jakimovska Özdemir (Acibadem University); Ugur Sezerman (Acibadem University); Luisa M Figueiredo (Instituto de Medicina Molecular)

14 (TT) Identifying molecular mediators of cellular states in *Trypanosoma cruzi*: comparison of single cell RNA sequencing technologies

Caroline D Keroack (Brown University); Sophie Marcus-Wade (Brown University); Jacqueline Lopez (Brown University); Eric D Salomaki (Brown University); Christopher L de Graffenried (Brown University)

15 (TT) LeishGEM: A genome-scale database for knockout mutant life cycle fitness phenotyping and subcellular protein localisation in *Leishmania mexicana*

Ulrich Dobramysl (University of Oxford); Eden R Ferreira (University of York); Rachel P Neish (University of York); Laura D Davidson (Oxford Brookes University); Raquel Pereira (University of Bern); Ruth Etzensperger (University of Bern); Sidonie Aellig (University of Bern); Matthew Young (University of Glasgow); James Smith (University of Glasgow); Jeziel Damasceno (University of Glasgow); Jack D Sunter (Oxford Brookes University); Jeremy Mottram (University of York); Eva Gluenz (University of Bern); Richard Wheeler (University of Oxford)

16 (TT) A novel conditional knockdown system in *T. cruzi* reveals essential roles for Aurora and Polo-like kinases in parasite cell division

Justin Wiedeman (University of Georgia); Ruby Harrison (University of Georgia); Drew Etheridge (University of Georgia)

17 (TT) A 2A peptide-based epitope-tagging toolkit for analysis of protein localisation, regulation, and function in Trypanosomatids

Carla Gilabert Carbajo (Imperial College London); Xiaoyang Han (Imperial College London); Bhairavi Savur (Imperial College London); Arushi Upadhyaya (Imperial College London); Fatima Taha (Imperial College London); Richard J Wheeler (University of Oxford); Michele Tinti (University of Dundee); Phillip Yates (Oregon Health and Science University); Calvin Tiengwe (Imperial College London)

25 (TT) The hydrophobic effect drives the outer-to-inner mitochondrial membrane connection of the Trypanosomal tripartite attachment complex

Philip Stettler (University of Bern); Salome Aeschlimann (University of Bern); Bernd Schimanski (University of Bern); Sandro Käser (University of Bern); André Schneider (University of Bern)

26 (TT) Bicarbonate – CO2 sensing and response mediated by trypanosome QIQ1

Anna Trenaman (University of Dundee); Federico Rojas (University of Edinburgh); Michele Tinti (University of Dundee); Keith Matthews (University of Edinburgh); Sam Alsford (London School of Hygiene and Tropical Medicine); David Horn (University of Dundee)

27 (TT) A genome-wide overexpression screen identifies proteins that inhibits the growth of *Trypanosoma brucei*

Navina Panneer Selvan (Rutgers University); Aditi Mukherjee (Rutgers University); Esteban Erben (IIBIO-UNSAM); Hee-Sook Kim (Rutgers University)

28 (TT) Host-pathogen dynamics of bumble bee parasite, *Crithidia bombi*

Blyssalyn V Bieber (Villanova University); Faith St. Clair (Villanova University); Sarah Lockett (Villanova University); Sonja Glasser (University of Massachusetts Amherst); Lynn S Adler (University of Massachusetts Amherst); Megan L Povelones (Villanova University)

67 A parasite's life cycle of RNA methylation

Lúcia Serra (Instituto de Medicina Molecular); Sara Silva-Pereira (Católica Biomedical Research Centre); Idálio J. Viegas (Institute of Inflammation and Ageing); Henrique Machado (Instituto de Medicina Molecular); Lara López-Escobar (Instituto de Medicina Molecular); Luisa M Figueiredo (Instituto de Medicina Molecular)

68 Characterization of a universally conserved histidine critical for tRNA deaminase activity

Aubree A Zimmer (Brown University, The Ohio State University); Luciano G Dolce (EMBL); Eva Kowalinski (EMBL); Diana Lopez (The Ohio State University); Marcos Sotomayor (The Ohio State University); Juan Alfonzo (Brown University)

69 Using an undergraduate research course to study of *Trypanosoma brucei* malate dehydrogenase isoforms using phosphomimetic mutations

Amy L Springer (University of Massachusetts, Amherst); Joseph J Provost (University of San Diego)

70 Glycosomal Fission in *Trypanosoma brucei*: The Critical Roles of TbDLP and TbFis1

Advaitha Iyer (DCBP, University of Bern); Silke Oeljeklaus (Biozentrum, University of Würzburg); Bettina Warscheid (Biozentrum, University of Würzburg); André Schneider (DCBP, University of Bern)

71 Dynamic Regulation of LmCOX4 and Metabolic Adaptation in *Leishmania* Under Mammalian Heat Stress

Isabel Stephany Brassesco (LSU Health New Orleans); Edward Wojcik (LSU Health New Orleans); Ben Kelly (LSU Health New Orleans)

72 Dynamics within the *Trypanosoma brucei* subpellicular microtubule array

Laura A Smithson (Brown University); Kevin E Ramrattan (Brown University); Emily K Campbell (Brown University); August Guang (Brown University); Christopher L de Graffenried (Brown University)

73 Investigating modulation of translation initiation in *Leishmania* parasites

Diana Calvopina Chavez (Harvard Medical School); Melissa Leger-Abraham (Harvard Medical School)

74 MPsome-RNA Helicase Nexus Governs Mitochondrial RNA Biogenesis in Trypanosomes

Takuma Suematsu (Boston University); Andres Vacas (Boston University); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine); Liye Zhang (Shanghai Tech University); Inna Afasizheva (Boston University); Ruslan Afasizhev (Boston University)

75 Polyadenylation of ribosomal RNA by *Trypanosoma cruzi* and *Trypanosoma brucei* complicates use of RNA sequencing technology

Caroline D Keroack (Brown University); Eric Salomaki (Brown University); Christopher L deGraffenried (Brown University)

76 Development of an Inducible Protein Degron System in *Trypanosoma brucei* and *Trypanosoma cruzi* to Facilitate Study of Essential Genes

Jacqueline Lopez (Brown University); Caroline Keroack (Brown University); Sophie Marcus-Wade (Brown University); Christopher L de Graffenried (Brown University)

77 Uncovering links between codon bias and tRNA expression dynamics in *Trypanosoma cruzi* life forms

Herbert Guimarães de Sousa Silva (Universidade Federal de São Paulo/Instituto Butantan); Satoshi Kimura (Cornell University College of Veterinary Medicine); Pedro Leonardo de Carvalho Lima (Instituto Butantan); David Pires (Instituto Butantan); Matthew K Waldor (Harvard Medical School/Brigham and Women's Hospital); Julia Pinheiro Chagas da Cunha (Universidade Federal de São Paulo/Instituto Butantan)

78 Direct demonstration that specific histone H4 tail lysines impact chromatin-based mechanisms in trypanosomes

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

79 Molecular dissection of VSG-Exclusion Protein 2 function and recruitment to the expression-site body

Sarah Stevens (University of York); Kiera Hayton (University of York); Josh Van Der Merwe (University of York); Beth Spink (University of York); Joana R Correia Faria (University of York)

80 A role for VSG mRNA in driving allelic competition

Douglas O Escrivani (University of Dundee); Michele Tinti (University of Dundee); Jane Wright (University of Dundee); Sebastian Hutchinson (Institut Pasteur, Paris); Anna Trenaman (University of Dundee); Joana R C Faria (University of York); David Horn (University of Dundee)

81 Characterization of TbTRF's TERRA binding and telomeric dsDNA binding activities

Lovlesh Thakur (Cleveland State University); Bibo Li (Cleveland State University)

82 de novo genome assembly of *Trypanosoma brucei* gambiense type 2 (Tbg2) and analysis of *T. brucei* subspecies population genetic structure

Mabel D. Tettey (Molecular Parasitology Section, Laboratory of Parasitic Diseases, NIAID, NIH); Natalia Grube (Molecular Parasitology Section, Laboratory of Parasitic Diseases, NIAID, NIH); Tom Hill (Integrated Data Sciences Section, Research Technology Branch, NIAID, NIH); Michael E. Grigg (Molecular Parasitology Section, Laboratory of Parasitic Diseases, NIAID, NIH)

83 Trypanosomatids and EdU: a good example of dose-response relationship

Marcos Antonio F de Oliveira (University of São Paulo); Vitor Luiz da Silva (University of São Paulo); Thaise L Teixeira (University of São Paulo); Bryan E Abuchery (University of São Paulo); Giovana Z Luduvice (University of São Paulo); Fernanda M Gerolamo (University of São Paulo); Marcelo S da Silva (University of São Paulo)

84 Cyclic AMP signaling impacts attachment of *Crithidia bombi*, a parasite of bumblebees, to surfaces in vitro

Sarah G Lockett (Villanova University); Blyssalyn V Bieber (Villanova University); Sonja K Glasser (University of Massachusetts Amherst); Neida O Portillo (University of Massachusetts Amherst); Faith A St. Clair (Villanova University); Madeline F Malfara (Villanova University); Michael Povelones (University of Pennsylvania); Lynn S Adler (University of Massachusetts Amherst); Megan L Povelones (Villanova University)

85 Structures of mitochondrial RNA editing helicase complexes

Shiheng Liu (University of California, Los Angeles); Takuma Suematsu (Boston University Medical Campus); Andres Vacas (Boston University Medical Campus); Inna Afasizheva (Boston University Medical Campus); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine); Xiaojing Zhao (ShanghaiTech University); Liye Zhang (ShanghaiTech University); Ruslan Afasizhev (Boston University Medical Campus); Z. Hong Zhou (University of California, Los Angeles)

86 A novel approach identifies transcriptome signatures across *Leishmania* spp. developmental stages and disease presentations

Ashton Trey Belew (University of Maryland, College Park); Najib El-Sayed (University of Maryland, College Park)

87 Depletion of a single IP6K allele leads *Trypanosoma cruzi* to a quiescence-like state

Bryan Etindi Abuchery (Universidade de São Paulo (USP)); Thaise Lara Texeira (Universidade de São Paulo (USP)); Vitor Luiz da Silva (Universidade de São Paulo (USP)); Bruno Alves Santarossa (Instituto Butantã); Miguel Antonio do Nascimento Garcia (Universidade Federal de São Paulo (UNIFESP)); Maria Cristina M. Motta (Universidade Federal de Rio de Janeiro (UFRJ)); Samuel C. Texeira (Universidade Federal de Uberlândia (UFU)); Nilmar S. Moretti (Universidade Federal de São Paulo (UNIFESP)); Eloisa A. V. Ferro (Universidade Federal de Uberlândia (UFU)); Simone Guedes Calderano (Instituto Butantã); Miguel A. Chiurillo (University of Cincinnati (UC)); Noelia M. Lander (University of Cincinnati (UC)); Roberto Docampo (University of Georgia (UoG)); Marcelo Santos da Silva (Universidade de São Paulo (USP))

88 The Tandem Zinc-fingers of KH2F1 and Putative Hydratase KH2F2 in the Helicase Complex REH2C Differentially Impact the Editing of Distinct Mitochondrial Transcripts in *Trypanosoma brucei* Development

Sunil K. Sharma (Texas A&M University); Tyler Rodshagen (Seattle Children's Research Institute); Cody Goode (Texas A&M University); Joshua Meehan (Texas A&M University); Lars O'Hara (Texas A&M University); Desmond Givens (Texas A&M University); Suzanne M. McDermott (Seattle Children's Research Institute); Jorge Cruz-Reyes (Texas A&M University)

Poster Session B

Thursday, September 12

7:00PM

35 (TT) High-accuracy genome assembly reveals hotspots for large gene families in *Trypanosoma cruzi* chromosomes

Lissa Cruz-Saavedra (McGill University); Luiza Berenguer Antunes (McGill University); Mira Loock (McGill University); Igor Cestari (McGill University)

36 (TT) Cross-linking and mass spectrometry uncovers chromatin compartment proteins and subtelomeric VSG gene silencing

Tony Isebe (McGill University); Luiza Antunes (McGill University); Oksana Kutova (McGill University); Igor Cestari (McGill University)

89 TblncRNA-23, a long non-coding RNA transcribed by RNA polymerase I, regulates developmental changes in *Trypanosoma brucei*

Beathrice Kostin-Galili (Bar-Ilan University); K Shanmugha (Weizmann Institute); Almog Freedman (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Smadar Chalamish-Cohen (Bar-Ilan University); Hiba Waldman Ben-Asher (Bar-Ilan University); Isabel Roditi (Bern University); Christian Tschudi (Yale University), Shulamit Michaeli (Bar-Ilan University)

90 Roles of *Trypanosoma brucei* POLIE and PPL2 in Telomere End Processing and Maintenance of Telomere Integrity

Prem P Kushwaha (Cleveland State University); Marjia Afrin (Cleveland State University); Elaina Casteel (Cleveland State University); Bibo Li (Cleveland State University)

91 Revisiting the impact of cruzipain inhibitor on *T. cruzi* egress

Sara De Grandis (Institute of Cell Biology); Anne Niggli (Institute of Cell Biology); Gaelle Lentini (Institute of Cell Biology)

92 Inositol pyrophosphates are involved in DNA metabolism, pathogenicity, and virulence in Trypanosomatids

Marcelo S. da Silva (University of São Paulo); Bryan E. Abuchery (University of São Paulo); Thaise L. Teixeira (University of São Paulo); Vitor L. da Silva (São Paulo State University); Suellen R. Maran (Federal University of São Paulo); Bruno A. Santarossa (Butantan Institute); Maria Cristina M. Motta (Federal University of Rio de Janeiro); Samuel C. Teixeira (Federal University of Uberlândia); Antônio M. Rezende (Oswaldo Cruz Foundation); Eloisa A.V. Ferro (Federal University of Uberlândia); Simone G. Calderano (Butantan Institute); Miguel A. Chiurrilo (University of Cincinnati); Noelia M. Lander (University of Cincinnati); Roberto Docampo (University of Georgia); Nilmar S. Moretti (Université de Montréal)

93 Discovery of an RNA binding complex from the antipodal nodes of *Trypanosoma brucei*

Andres F Vacas (Boston University); Takuma Suematsu (Boston University); Clinton Yu (University of California, Irvine); Xiaojing Zhao (ShanghaiTech University); Fan Zhang (ShanghaiTech University); Lan Huang (University of California, Irvine); Liye Zhang (ShanghaiTech University); Inna Afasizheva (Boston University)

94 LeishTag: Defining the subcellular localisations of the *Leishmania* specific proteome

Laura Davidson (Oxford Brookes University); Ulrich Dobramysl (University of Oxford); Jeremy Mottram (University of York); Eva Gluenz (Univeristy of Bern); Richard J Wheeler (University of Oxford); Jack D Sunter (Oxford Brookes University)

95 The Role of Haptoglobin-Related Protein in Innate Immunity to African Trypanosomes

Amar S Dhanjal (Macaulay Honors College, Hunter College); Sara Fresard (Hunter College); Jayne Raper (Hunter College)

96 Determining the role of sodium accumulation in TLF-mediated lysis of African Trypanosomes

Arva Demaliaj (Department of Biological Sciences, Hunter College-CUNY); Sara Fresard (Department of Biological Sciences, Hunter College-CUNY; Biology Program, The Graduate Center CUNY); Jayne Raper (Department of Biological Sciences, Hunter College-CUNY; Biology Program, The Graduate Center CUNY)

97 Investigating the Interaction of TbICP and TbCatL Recombinant Protein in a CHO-S Mammalian model

Milany Bruno (CUNY Hunter College); Bernardo Gonzalez-Baradat (CUNY Hunter College); Daniel Lopes (CUNY Hunter College); Jayne Raper (CUNY Hunter College)

98 Elucidating ICP Localization in *Trypanosoma brucei*

Nyhal Metidji (CUNY Hunter College); Bernardo Gonzalez-Baradat (CUNY Hunter College); Zijing Cao (CUNY Hunter College); Daniel Lopes (CUNY Hunter College); Jayne Raper (CUNY Hunter College)

99 DNA replication compartmentalisation dictates genome stability and instability in *Trypanosoma brucei*

Marija Krasilnikova (University of Glasgow); Catarina A Marques (University of Glasgow); Emma M Briggs (University of Edinburgh); Craig Lapsley (University of Glasgow); Graham Hamilton (University of Glasgow); Dario Beraldi (University of Glasgow); Kathryn Crouch (University of Glasgow); Richard McCulloch (University of Glasgow)

100 Trypanosomatid parasites as a model for termination of translation and stop codon read-through

Julie Kovarova (Institute of Parasitology); Nathalia Ballesteros Chitiva (Institute of Parasitology); Zdenek Paris (Institute of Parasitology)

101 Role of the RNA binding protein RBP42 in *Trypanosoma brucei* bloodstream form parasites

Anish Das (Rutgers, New Jersey Medical School); Tong Liu (Rutgers, New Jersey Medical School); Hong Li (Rutgers, New Jersey Medical School); Seema Husain (Rutgers, New Jersey Medical School)

102 Can we visualize Apolipoprotein L1 at the plasma membrane of African Trypanosomes?

Kayla Leiss (Department of Biological Sciences, Hunter College-CUNY); Sara Fresard (Biology Program, The Graduate Center CUNY); Jayne Raper (Department of Biological Sciences, Hunter College-CUNY)

103 TbICP, where are you? Intracellular localization of the Inhibitor of the Cathepsin L of *Trypanosoma brucei*

Bernardo Gonzalez (Hunter College); Nyhal Metidji (Hunter College); Kayla Leiss (Hunter College); Daniel Lopes (Hunter College); Jayne Raper (Hunter College)

104 tRNATyr Has an Unusually Short Half-Life in *Trypanosoma brucei*

Gabriel Silveira d'Almeida (Brown University); Ananth Casius (Brown University); Jeremy C Henderson (New England Biolabs); Sebastian Knuesel (Boston University); Ruslan Afasizhev (Boston University); Inna Afasizheva (Boston University); Aidan C Manning (University of California, Santa Cruz); Todd M Lowe (University of California, Santa Cruz); Juan D Alfonzo (Brown University)

105 To ligate or not to ligate: A splicing-independent function for the essential TRL1 homolog in *Trypanosoma brucei*.

Ananth Casius (The Ohio State University, Brown University); Lankani Gunaratne (The Ohio State University, Brown University); Christopher L de Graffenried (Brown University); Juan D. Alfonzo (Brown University)

106 Exploring the role of KREPA6 and other OB-fold proteins in RNA editing

Brittney Davidge (Seattle Children's Research Institute); Suzanne McDermott (Seattle Children's Research Institute; University of Washington School of Medicine Department of Pediatrics); Jason Carnes (Seattle Children's Research Institute); Tyler Rodshagen (Seattle Children's

Research Institute); Isaac Lewis (Seattle Children's Research Institute); Maxwell Tracy (Seattle Children's Research Institute. Present address: UC Davis Integrative Pathobiology Program); Kenneth D. Stuart (Seattle Children's Research Institute; University of Washington School of Medicine Department of Pediatrics)

107 TcVPS23: ESCRT I complex acts in the EV biogenesis pathway and in immunopathology of *Trypanosoma cruzi* experimental infection

Nadjania Saraiva de Lira Silva (Universidade Federal de São Paulo); Ana Claudia Trocoli Torrecilhas (Universidade Federal de São Paulo); Sergio Schenkman (Universidade Federal de São Paulo)

108 KRBP72 facilitates ATPase-dependent editing progression through a structural roadblock in ATPase subunit 6 mRNA

Ashutosh P Dubey (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA); Brianna L Tylec (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA); Soon Y Yi (Center for RNA Science and Therapeutics, School of Medicine, Case Western Reserve University, Cleveland, OH, USA. Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH, USA.); Frank A Tedeschi (Center for RNA Science and Therapeutics, School of Medicine, Case Western Reserve University, Cleveland, OH, USA. Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH, USA.); Joseph T Smith (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA); Laurie K Read (Dept. of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA)

109 Functional significance of DRBD18 in mitochondrial RNA editing of *Trypanosoma brucei*

Parul Pandey (Jacobs School of Medicine and Biomedical Science, University at Buffalo); Katherine Wackowski (Jacobs School of Medicine and Biomedical Science, University at Buffalo); Ashutosh P. Dubey (Jacobs School of Medicine and Biomedical Science, University at Buffalo); Laurie K. Read (Jacobs School of Medicine and Biomedical Science, University at Buffalo)

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

Katherine Wackowski (Department of Microbiology and Immunology, University at Buffalo); Xiaoyu Zhu (Department of Pharmaceutical Sciences, University at Buffalo); Shichen Shen (Department of Pharmaceutical Sciences, University at Buffalo); Ming Zhang (Department of Pharmaceutical Sciences, University at Buffalo); Jun Qu (Department of Pharmaceutical Sciences, University at Buffalo); Laurie K Read (Department of Microbiology and Immunology, University at Buffalo)

111 Analysis of introns in kinetoplastids: a novel intron-containing gene and the first known eukaryote with only trans-splicing

Alexei Yu Kostygov (University of Ostrava); Karolína Skýpalová (University of Ostrava); Natalia Kraeva (University of Ostrava); Elora Kalita (University of Ostrava); Cameron McLeod (University of Dundee); Vyacheslav Yurchenko (University of Ostrava); Mark C Field (University of Dundee); Julius Lukeš (Institute of Parasitology, Czech Academy of Sciences); Anzhelika Butenko (Institute of Parasitology, Czech Academy of Sciences)

112 Understanding the consequences of a possible crosstalk between inositol pyrophosphates (PP-IPs) and telomeric dynamics in *Leishmania braziliensis*

Vitor Luiz da Silva (São Paulo State University "Júlio de Mesquita Filho"); Thaise Lara Teixeira (São Paulo University); Suellen Rodrigues Maran (Federal University of São Paulo); Nilmar Moretti (Faculté de Medicine Vétérinaire); Antônio Mauro Rezende (Oswaldo Cruz Foundation); Marcelo Santos da Silva (São Paulo University)

113 Molecular Insights into Immune and Tissue Repair Mechanisms Associated with Congenital Chagas

Sneider Alexander Gutierrez Guarnizo (Johns Hopkins University); Jill Hakim (Johns Hopkins University); Carolina Duque (Johns Hopkins University); Jessi Condori Samame (Universidad Peruana Cayetano Heredia); Paloma Samame (Hospital de La Mujer Dr. Percy Boland Rodriguez); Emily Arteaga (Hospital de La Mujer Dr. Percy Boland Rodriguez); Jean Karla Velarde (Hospital de La Mujer Dr. Percy Boland Rodriguez); Alejandra Diestra (Universidad Peruana Cayetano Heredia); Alejandra Pando (Universidad Peruana Cayetano Heredia); Monica Pajuelo (Universidad Peruana Cayetano Heredia); Manuela Verastegui (Universidad Peruana Cayetano Heredia); Freddy Tinajeros (Hospital de La Mujer Dr. Percy Boland Rodriguez); Natalie Bowman (University of North Carolina); Robert Gilman (Johns Hopkins University); Monica Mugnier (Johns Hopkins University)

114 Profiling the human IgG response to *T. brucei* spp. infections using a Pan-VSG phage display library

Jaime So (Johns Hopkins SChool of Public Health); Bailin Zhang (Johns Hopkins School of Public Health); Louise Singer (Johns Hopkins School of Public Health); Monica Mugnier (Johns Hopkins School of Public Health)

115 *Leishmania* amazonensis aquaglyceroporin 1 is a glycosomal protein involved in antimonial resistance

Romario Lopes Boy (Department of Physiology, Institute of Biosciences, University of São Paulo (USP)); Ricardo Andrade Zampieri (Department of Physiology, Institute of Biosciences, University of São Paulo (USP)); Juliana Ide Aoki (Department of Animal Biology, Institute of

Biology, State University of Campinas (UNICAMP)); Lucile Maria Floeter Winter (Department of Physiology, Institute of Biosciences, University of São Paulo (USP)); Maria Fernanda Laranjeira Silva (Department of Physiology, Institute of Biosciences, University of São Paulo (USP))

116 Amino Acid Permease 3 senses arginine availability and responds to temperature and pH changes in *Leishmania major*

Ricardo Andrade Zampieri (University of São Paulo); Romario Lopes Boy (University of São Paulo); Juliana Ide Aoki (State University of Campinas); Maria Fernanda Laranjeira Silva (University of São Paulo); Karl Erik Muller (Oslo University Hospital); Audun Helge Nerland (University of Bergen); Lucile Maria Floeter Winter (University of São Paulo)

117 Translation of the non-canonical genetic code of *Blastocrithidia* nonstop

Julie Kovářová (Institute of Parasitology, Biology Centre, Czech Academy of Science); Nathalia Ballesteros Chitiva (Institute of Parasitology, Biology Centre, Czech Academy of Science); Zdeněk Paris (Institute of Parasitology, Biology Centre, Czech Academy of Science)

Poster Session C

Friday, September 13

7:00PM

45 (TT) Genome analysis of *T. cruzi* field isolates offers the opportunity to study the effect of infection context on parasite genetic diversity

Jill Hakim (Johns Hopkins); Sneider Gutiérrez (Johns Hopkins); Edith Malaga (Universidad Cayetano Herida); Louisa Messenger (University of Arizona); Monica Mugnier (Johns Hopkins)

46 (TT) Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models

Sara Fresard (CUNY Hunter College, The Graduate Center at City University of New York); Kayla Leiss (CUNY Hunter College); Russell Thomson (CUNY Hunter College); Jayne Raper (CUNY Hunter College, The Graduate Center at City University of New York)

47 (TT) Host response to cutaneous Human African Trypanosomiasis

Rhiannon Heslop (University of Glasgow); Nono-Raymond Kuispond Swar (National Institute for Biomedical Research, Kinshasa); Anneli Cooper (University of Glasgow); Paul Capewell (University of Glasgow); Dieudonne Mumba Ngoyi (National Institute for Biomedical Research, Kinshasa); Annette MacLeod (University of Glasgow)

55 (TT) RNA viruses in *Blastocrithidia*: does a unique genetic code protect from viral infection?

Danyil Grybchuk (University of Ostrava); Donnamae Klocek (University of Ostrava); Alexei Kostygov (University of Ostrava); Vyacheslav Yurchenko (University of Ostrava)

56 (TT) REH2C Complex is the First Identified Regulatory Factor in Lifecycle Stage-specific RNA Editing Repression During *Trypanosoma brucei* Development

Cody Goode (Texas A&M University); Binny Kaur (Texas A&M University); Sunil Sharma (Texas A&M University); Zihao Chen (University of Edinburgh); Tyler Rodshagen (Seattle Children's Research Institute); Scott Grote (Harvard Medical School); Joshua Meehan (Texas A&M University); Al Ivens (University of Edinburgh); Nick J. Savill (University of Edinburgh); Silvi Rouskin (Harvard Medical School); Achim Schnaufer (University of Edinburgh); Suzanne M. McDermott (Seattle Children's Research Institute); Jorge Cruz-Reyes (Texas A&M University)

57 (TT) Deep mutational resistance profiling for anti-Trypanosomal proteasome inhibitors

Simone Altmann (University of Dundee); Michele Tinti (University of Dundee); Melanie Ridgway (University of Dundee); Manu de Rycker (University of Dundee); Michael Thomas (University of Dundee); Cesar Mendoza Martinez (University of Dundee); Jagmohan Saini (University of Dundee); Peter Ibrahim (University of Dundee); Mike Bodkin (University of Dundee); David Horn (University of Dundee)

58 (TT) Forging the One Ring of *Trypanosoma brucei*: Structural characterization of BILBO1 assembly

Kim Ivan Abesamis (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter); Arda Kara (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter); Derrick Robinson (Microbiologie Fondamentale et Pathogénicité, CNRS UMR5234, University of Bordeaux); Mélanie Bonhivers (Microbiologie Fondamentale et Pathogénicité, CNRS UMR5234, University of Bordeaux); Gang Dong (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter)

118 Endocytosis and Recycling of the Surface Protein Coat in *Trypanosoma brucei*

Adamu Musa Muhammed (Department of Cell & Developmental Biology, Biocenter, University of Würzburg, Würzburg, Germany); Fabian Link (Department of Cell & Developmental Biology, Biocenter, University of Würzburg, Würzburg, Germany); Thomas Müller (Department of Cell & Developmental Biology, Biocenter, University of Würzburg, Würzburg, Germany); Kevin Bongers (Department of Cell & Developmental Biology, Biocenter, University of Würzburg, Würzburg, Germany); Markus Engstler (Department of Cell & Developmental Biology, Biocenter, University of Würzburg, Würzburg, Germany)

119 Exploring the secretory pathway in African trypanosomes via proximity labelling

Siqi Shen (Charles University); Martin Zoltner (Charles University)

120 The protein kinase ATAXIA-TELANGIECTASIA AND RAD3-RELATED (ATR) is an important player to guarantee the genome integrity in *Leishmania major*.

Gabriel da Silva (University of Glasgow); Jeziel Damasceno (University of Glasgow); Jennifer Black (University of Sao Paulo); Luiz Tosi (University of Sao Paulo); Richard McCulloch (University of Glasgow)

121 TbKAP7/HMG3 is essential for growth and cell division in *Trypanosoma brucei*

Johannes P. Maree (Villanova University); Olivia R. Picca (Villanova University); Megan L. Povelones (Villanova University)

122 A novel family of SET domain protein lysine methyltransferases is essential for mitochondrial function in *Trypanosoma brucei*.

Emily Knight (Clemson University); Devon McCourry (Indiana University School of Medicine); Evan Cornett (Indiana University School of Medicine); Meredith Morris (Clemson University)

123 High-throughput cell-cycle synchronisation of *Leishmania mexicana*

Elizabeth Thwaites (Lancaster University); Chloe Barnes (Lancaster University); Richard Burchmore (Glasgow University); Richard McCulloch (Glasgow University); Michael D. Urbaniak (Lancaster University)

124 A triple inducible system to study the role of POLIB in kDNA replication and DNA damage response

Raveen Armstrong (University of Massachusetts Amherst); Matthew Romprey (University of Massachusetts Amherst); Henry Raughley (University of Massachusetts Amherst); Stephanie Delzell (University of Massachusetts Amherst); Matthew Frost (University of Massachusetts Amherst); David Anaguano (University of Massachusetts Amherst); James Chambers (University of Massachusetts Amherst); Michele Klingbeil (University of Massachusetts Amherst)

125 Mechanisms by which African trypanosomes sense and respond to iron availability

Harsh A Pawar (Lancaster University); Chloe Barnes (Lancaster University); Eve Lancaster (Lancaster University); Michael D Urbaniak (Lancaster University)

126 A NEK protein kinase family member is involved in the differentiation and kinetoplast division of *Trypanosoma cruzi*

Jessica Huckleberry (University of Cincinnati); Juliana Roson (University of Cincinnati); Noelia Lander (University of Cincinnati); Miguel Chiurillo (University of Cincinnati)

127 Investigating the sequence homology requirements for mosaic VSG formation in *Trypanosoma brucei*

Erin M Kennedy (Johns Hopkins Bloomberg School of Public Health); Jaclyn E Smith (Johns Hopkins Bloomberg School of Public Health); Kevin J Wang (Johns Hopkins Bloomberg School of Public Health); Monica R Mugnier (Johns Hopkins Bloomberg School of Public Health)

128 SLAM-seq reveals independent contributions of RNA processing and stability to gene expression in African trypanosomes

Vanessa Luzak (LMU Munich); Esteban Osses (LMU Munich); Anna Danese (LMU Munich); Christoff Odendaal (Vrije Universiteit Amsterdam, Amsterdam); Stefan H Stricker (LMU Munich); Jurgen R Haanstra (Vrije Universiteit Amsterdam, Amsterdam); Florian Erhard (University of Regensburg); T. Nicolai Siegel (LMU Munich)

129 Quantitative proteomic analysis reveals different responses dependent on the SLAMF1 receptor and the *Trypanosoma cruzi* strain

Alfonso Herreros-Cabello (CBMSO (CSIC-UAM)); Javier del Moral-Salmoral (CBMSO (CSIC-UAM)); Esperanza Morato (CBMSO (CSIC-UAM)); Anabel Marina (CBMSO (CSIC-UAM)); Beatriz Barrocal (CBMSO (CSIC-UAM)); Manuel Fresno (CBMSO (CSIC-UAM)); Núria Gironès (CBMSO (CSIC-UAM))

130 Transcriptomic analysis in macrophages infected with *Trypanosoma cruzi*

Francisco Callejas-Hernández (CBMSO (CSIC-UAM)); Alfonso Herreros-Cabello (CBMSO (CSIC-UAM)); Cristina Poveda (CBMSO (CSIC-UAM)); María C Maza (CBMSO (CSIC-UAM)); Diana K Santos-Peñaloza (CBMSO (CSIC-UAM)); Manuel Fresno (CBMSO (CSIC-UAM)); Núria Gironès (CBMSO (CSIC-UAM))

131 Inducible Genome-Scale Overexpression and RNAi libraries for Genetic Screens in *Leishmania* species

Corinne M Fargo (Oregon Health & Science University); Galadriel A Hovel-Miner (George Washington University); Tiago R Ferreira (National Institute of Allergy and Infectious Diseases, NIH); Alejandro Sanchez-Salvador (Universidad Autonoma de Madrid); Jose M Requena (Universidad Autonoma de Madrid); Sloane M Domico (Oregon Health & Science University); Lon-Fye Lye (Washington University School of Medicine); Stephen M Beverley (Washington University School of Medicine); Phillip A Yates (Oregon Health & Science University)

132 Potential roles for RNA modification in *Trypanosoma brucei* mitochondrial RNA editing

Suzanne McDermott (Seattle Children's Research Institute); Vy Pham (Seattle Children's Research Institute); Isaac Lewis (Seattle Children's Research Institute); Tyler Rodshagen (Seattle Children's Research Institute); Maxwell Tracy (Seattle Children's Research Institute); Ken Stuart (Seattle Children's Research Institute)

133 Growth and RNA-seq of micropopulations of *T. brucei* in thousands of semi-permeable capsules

Anna Barcons (LMU Munich); Kiryl Kavaliou (LMU Munich); Nicolai Siegel (LMU Munich); Esteban Osses (LMU Munich)

134 Identification of cell cycle regulated genes as potential nuclear DNA replication players in *Trypanosoma brucei*

Grace Gill (University Of Glasgow); Catarina A Marques (University Of Glasgow); Emma M Briggs (University of Edinburgh); Richard McCulloch (University Of Glasgow)

135 Benzoxaborole AN15368 induces DNA damage and synergizes with DNA repair inhibitors to kill intracellular *Trypanosoma cruzi*

Aylla S K Von Ermland (Center for Tropical and Emerging Global Diseases, University of Georgia); Jihyun Lim (Center for Tropical and Emerging Global Diseases, University of Georgia); Fernando Sanchez-Valdéz (Center for Tropical and Emerging Global Diseases, University of Georgia); José L Saenz (Center for Tropical and Emerging Global Diseases, University of Georgia); Rick L Tarleton (Center for Tropical and Emerging Global Diseases, University of Georgia)

136 Exploring structural features of a potential fatty acid binding protein of *Leishmania* amazonensis

Lucas Athayde (University of São Paulo); Marco Gomes (University of São Paulo); Simona Tomaselli (Istituto di Scienze e Tecnologie Chimiche Giulio Natta); Maria Fernanda Laranjeira Silva (University of São Paulo); Roberto Kopke Salinas (University of São Paulo)

137 Unraveling the Enigmatic Feeding Apparatus of *Trypanosoma cruzi*: Using Proximity Labeling to Identify the Molecular Components of the Cytostome-Cytopharynx Complex

Gonzalo Seminario-Mondejar (UGA); Ronald Drew Etheridge (UGA)

138 Affinity purification of Kinetoplastea mitochondria

Md Solayman (Boston University); Andres Vacas (Boston University); Inna Afasizheva (Boston University); Ruslan Afasizhev (Boston University)

139 Elucidating the Organization of Intraflagellar Transport at the Flagellum Base in *Trypanosoma brucei*

Sophia R Staggers (University of Pittsburgh); Muyuan Chen (SLAC National Accelerator Laboratory, Stanford University); Zhangyu (Sharey) Cheng (Carnegie Mellon University Mellon College of Science); Yongxin (Leon) Zhao (Carnegie Mellon University Mellon College of Science); Stella Y Sun (University of Pittsburgh)

140 Cryo-Electron Tomography Analysis of Nuclear Envelope Dynamics During "Closed" Mitosis in *Trypanosoma brucei*

Mingqi Zhao (University of Pittsburgh); Sophia R. Staggers (University of Pittsburgh); Stella Y. Sun (University of Pittsburgh)

ABSTRACTS

Session I – Abstracts

Parasites: sensing and responding

Chair: Calvin Tiengwe

Tuesday, September 10

7:00PM – 9:00PM

1 Single-cell transcriptomic heterogeneity of *Leishmania major* transmission and development in their natural sand fly vector

Carolina M.C. Catta-Preta (NIAID, NIH); Kashinath Ghosh (NIAID, NIH); David L. Sacks (NIAID, NIH); Tiago R. Ferreira (NIAID, NIH)

Leishmania development in the sandfly vector has been predominantly characterized as a series of sequential promastigote morphotypes. The absence of validated molecular markers for the vast majority of *Leishmania* developmental forms represents a significant obstacle in the field. Beyond the well-characterized mammal-infective metacyclics, the gene expression profiles of promastigotes colonizing the sand fly gut or transmitted to the host remain under-explored. In this study, by using single-cell RNA-sequencing (scRNA-seq), we investigated the transcriptomic landscape of *Leishmania major* inside their natural vector, Phlebotomus duboscqi, at early infection (days 2 and 5 post-blood meal), at late infection and in the transmitted dose (day 9). We identified markers for three distinct subpopulations in sand flies harboring mature, transmissible infections at day 9. These markers were validated by endogenous gene tagging with fluorescent reporters, which highlighted the location and morphology of stages forming a mature infection. Our pseudotime analysis revealed a non-linear developmental trajectory starting at day 5, which split upon reaching the leptomonad stage into either attached/detached haptomonads or dividing early-metacyclics, an undescribed stage. At day 9, a trajectory progressed from early-metacyclics to classical highly motile, non-dividing metacyclics, here renamed late-metacyclics. We further described that detached haptomonads are part of the transmitted inoculum, along with late-metacyclics. FACS-sorted haptomonads are infectious and capable of exacerbating pathology in BALB/c mice, suggesting their contribution to vector infectivity. Our scRNA-seq analysis and the identification of novel markers provide a new framework for studying the *Leishmania* life cycle and its impact on both transmission and host infection.

2 A single cell RNA sequencing atlas of *Trypanosoma cruzi* development reveals high resolution heterogeneity in the parasite lifecycle

Ross F Laidlaw (University of Glasgow); Marta Garcia-Sanchez (University of Dundee); Juliana Da Silva Pacheco (University of Dundee); Luciana De Sousa Paradela (University of Dundee); Thomas D Otto (University of Glasgow); Manu De Rycker (University of Dundee)

Single cell RNA-seq (scRNA-seq) has already proved its capacity to enrich the knowledge on the transcriptomic changes that mark and govern the lifecycle of parasites. Of the three disease causing Trypanosomatids: *Trypanosoma brucei* (*T. brucei*), *Leishmania* & *Trypanosoma cruzi* (*T. cruzi*), *T. cruzi* stands alone with no published scRNA-seq datasets available to the community. We present not only the first scRNA-seq dataset of *T. cruzi*, but the first cell atlas for the in vitro *T. cruzi* lifecycle, with single cell transcriptomes captured for over 31,000 cells. We validated the atlas using known life-cycle stage markers and population-based transcriptomics. The atlas reveals many life-cycle stage associated genes and key transcriptomic differences between two amastigote-derived trypomastigote subtypes. Enabled by single cell resolution, we define the transcriptomic changes that occur across the epimastigote to metacyclic trypomastigote differentiation axis and identify several differentially expressed RNA binding protein encoding genes which have not previously been associated with metacyclogenesis in *T. cruzi*. Finally, we show that the atlas can be utilised to accurately annotate lifecycle stage in novel data, providing a baseline for the community when annotating their own scRNA-seq datasets. This atlas provides not only a comprehensive resource for the study of *T. cruzi* biology, but also an unprecedented look at the complex changes that occur across the parasite lifecycle.

3 Hide and Go Seq: Capturing the Antibody-VSG Arms Race During *Trypanosoma brucei* Infection

Lulu M Singer (Johns Hopkins Bloomberg School of Public Health); Jaime E So (Johns Hopkins University School of Medicine); Alexander K Beaver (Johns Hopkins University School of Medicine); Monica R Mugnier (Johns Hopkins Bloomberg School of Public Health)

Trypanosoma brucei, the protozoan parasite that causes Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT), continues to pose significant medical and economic burdens in endemic countries. *T. brucei*, an entirely extracellular parasite, is able to evade clearance using a sophisticated mechanism of antigenic variation. The parasite is covered in a dense coat of the antigenically variable Variant Surface Glycoprotein (VSG) and can switch expression of its VSG to avoid elimination by antibodies. *T. brucei* has access to a genomic repertoire of 1000s of VSG encoding genes that can be expressed or recombined into novel VSGs, giving the parasite a massive pool of antigens to choose from. With potentially hundreds of VSGs expressed during a single infection, the anti-VSG response is impossible to capture using standard low throughput techniques, and little is known about the in vivo anti-VSG response. Here, we aimed to elucidate the dynamics of the VSG-antibody interface during *T. brucei* infection in mice using high throughput methods. We combined VSG-seq, a targeted mRNA sequencing approach, with phage immunoprecipitation sequencing (PhIP-seq), a high throughput epitope mapping method. Using a phage display library of over 76,000 VSG peptides, we were able to track the dynamics and specificities of the anti-VSG response. With this longitudinal approach, we were able to visualize the isotype specific kinetics of the anti-VSG response during infection. We were also able to identify and map peptide epitopes to expressed VSGs, providing insight into binding specificities and what VSG regions are accessible to different isotypes.

4 Nutritional stress as a signal for *Trypanosoma brucei* differentiation to the mesocyclic-like form

Eva Dolezelova (Biology Centre, CAS); Hana Pavliskova (Institute of Molecular Genetics, CAS); Tomas Skalicky (Biology Centre, CAS); Martin Moos (Biology Centre, CAS); Vladimir Varga (Institute of Molecular Genetics, CAS); Alena Zikova (Biology Centre, CAS)

The journey of *Trypanosoma brucei* through the tsetse fly's alimentary tract involves complex differentiation through various life cycle forms. Following the establishment of a midgut infection, procyclic form (PF) cells migrate to the proventriculus, which is associated with their differentiation into mesocyclic cells, characterised by an elongated shape, swift swimming, and synchronised cell cycle arrest. The environmental and intracellular triggers for this differentiation remain largely unknown. Our findings demonstrate that PF cells at stationary phase in culture or with RNAi-suppressed function of the ribose-5-phosphate isomerase (TbRPI), a pentose phosphate pathway enzyme that provides substrate for nucleotide synthesis, exhibit morphological and behavioral traits that are highly reminiscent of mesocyclic parasites. TbRPI is localised in the cytosol and at the flagellum tip suggesting a metabolic and sensory role. Comparative analysis revealed significant similarities in the transcriptomic and proteomic landscape between TbRPI-suppressed cells and stationary parasites. Both conditions upregulate proteins for cell motility and vesicle transport, while downregulating those for translation and chromosome segregation. Metabolomic analysis indicated nucleotide metabolism as a potential trigger for the morphological features and cell cycle arrest in TbRPI-suppressed cells. The phenotype caused by TbRPI suppression was reversed by the introduction of RPIs from *T. cruzi* and E. coli, but not by expression of TbRPI with a mutated amino acid in the enzyme's active site, indicating that the phenotype is caused by the lack of the RPI enzymatic activity. Our findings suggest a direct link between impaired nucleotide synthesis and parasite differentiation to mesocyclic-like cells induced by nutritional stress.

5 Proteomic and lipidomic profiles reveal phospholipase-dependent adaptation to heat-stress in African trypanosomes

Gustavo Bravo Ruiz (University of Dundee); Rupa Nagar (University of Dundee); Michele Tinti (University of Dundee); Michael Ferguson (University of Dundee); David Horn (University of Dundee)

African trypanosomes experience temperature fluctuations during transfer between the tsetse insect vector and mammalian hosts, due to diurnal shift, or due to fever symptomatic of human infection. The Trypanosomal response to heat-shock has not been described in any detail at the proteomic or lipidomic level, however. We used state-of-the-art proteomic analysis to quantify >5,900 proteins in bloodstream form African trypanosomes that are typically grown at 37°C, after growth at 34°C, 37°C or 40°C for six hours. The analysis revealed cytoplasmic, endoplasmic reticulum, and mitochondrion-localised heat shock proteins, surface proteins, (co-)chaperones, proteases, protein isomerases, and RNA-binding proteins for which abundance was significantly correlated with temperature. Among the upregulated proteins, we found a phospholipase encoded by a horizontally transferred PLA1 gene, suggesting a role in membrane remodelling. We used CRISPR-Cas9 to assemble pla1 null strains, which were hyper-sensitive to heat-shock. Lipidomic analysis revealed a temperature-dependent increase in the abundance of saturated fatty acids, consistent with homeoviscous adaptation, while pla1 strains displayed a defect in this response. We also identify sequences in 3'-untranslated regions that likely drive the post-transcriptional heat-shock response. Thus, we provide a proteomic profile of the post-transcriptional temperature sensing and heat-shock response in the African trypanosome. We also conclude that PLA1 is required for phospholipid remodelling and rapid adaptation to increased temperature in these important parasites.

6 *Leishmania* ESB1 ortholog regulates delta amastin expression

Jorge Arias del Angel (Oxford Brookes University); Richard J Wheeler (University of Edinburgh); Jack D Sunter (Oxford Brookes University)

The major *Leishmania* cell coat components are amastin and GP63 proteins, and abundant lipophosphoglycans and glycoinositolphospholipids. The delta amastin family is associated with the evolution of mammalian parasitism and are upregulated in the mammalian amastigote form. Specific 3' UTR elements and mRNA binding proteins have been identified that regulate life cycle stage-specific amastin mRNA stability. In contrast, surface coat protein expression in *Trypanosoma brucei* is regulated using stage-specific RNA polymerase I promoters – tightly regulated, in the bloodstream form, for monoallelic expression of VSG coat proteins. We recently discovered ESB1 as necessary for VSG expression, and transcriptional activation of the VSG expression site. Intriguingly, all Trypanosomatid parasites encode an ESB1 ortholog. Here, we analyse the function of the *Leishmania mexicana* ortholog of ESB1. LmESB1 is a nuclear protein, localising to nucleoplasm foci but not colocalising with RNA Pol I. Deletion of LmESB1 caused slowed growth and upregulation of delta amastin and tuzin transcripts from a single genomic locus. However, this phenotype was not stable, and selection for fast growth through promastigote culture led to a recovery of growth rate and reversion of amastin and tuzin transcript abundance to parental levels. This transcriptomic analysis identified changes associated with reversion of the amastin and tuzin transcript levels. This included a downregulated putative RNA binding protein and an upregulated RNA polymerase II regulator, and deletion of either resulted in the upregulation of an overlapping set of delta amastin transcripts from the various delta amastin loci. Overall, our work has begun to delineate a novel regulatory network controlling delta amastin expression in *Leishmania*, and indicates nuclear mechanisms involving ESB1 have a role in surface coat regulation in diverse Trypanosomatids.

7 Genome-wide fitness profiling by DRiF-Seq identifies novel regulators acting early in the *Trypanosoma brucei* quorum sensing signalling pathway

Kirsty R McWilliam (The University of Edinburgh); Simon D'Archivio (The University of Nottingham); Phoebe Kim (The University of Edinburgh); Olga Dluzniewska (The University of Edinburgh); Catarina Gadelha (The University of Nottingham); Keith R Matthews (The University of Edinburgh)

Within mammalian hosts, *Trypanosoma brucei* undergoes a density-dependent developmental transition from proliferative 'slender' cells, that establish infection, to cell-cycle arrested 'stumpy' cells competent for transmission to tsetse flies. This quorum sensing (QS)-like process is triggered by an increasing concentration of extracellular oligopeptides transported into the cell via the parasite surface transporter, TbGPR89. An earlier genome-wide RNAi screen identified molecules that form part of the *T. brucei* QS signal transduction pathway (Mony, MacGregor et al, Nature, 2014). However, since this screen used cell-permeable cAMP to activate the pathway in laboratory-adapted parasites that are unresponsive to the natural QS signal, TbGPR89 and the molecules immediately downstream of the transporter were bypassed. Thus, the molecules that operate in the earliest steps in the QS signalling pathway remain unknown. To identify these molecules we have used Direct RNAi Fragment Sequencing, DRiF-Seq, to perform a genome-wide quantitative fitness profiling in QS-responsive pleomorphic parasites exposed to the physiological oligopeptide differentiation signal. Validating the screen, previously known quorum-sensing components were identified, along with many novel genes. Two novel kinases identified by the screen, AKT-like kinase and an AGCK family kinase, were explored in depth, demonstrating that knock-down, knock-out or targeted mutagenesis of either kinase reduced slender to stumpy differentiation both in vitro and in vivo. Importantly, we positioned the molecules in the QS-signalling pathway, demonstrating that these kinases operate upstream of all previously identified QS signal transduction pathway components except TbGPR89. These results provide important new insight into the earliest signalling events controlling *T. brucei* quorum-sensing.

8 (TT) Decoding single cell gene expression as parasites invade the adipose tissue

Lara López-Escobar (Instituto de Medicina Molecular); Milena Jakimovska Özdemir (Acibadem University); Ugur Sezerman (Acibadem University); Luisa M Figueiredo (Instituto de Medicina Molecular)

Trypanosoma brucei is a motile parasite that invades and adapts to organs, including adipose tissue. To explore parasite heterogeneity during mouse infection, we developed a 10x Genomics single-cell RNA sequencing (scRNAseq) protocol to capture parasites from blood and adipose tissue at the onset of tissue colonization. Our findings reveal that adipose tissue is initially colonized by slender forms in the G1 cell cycle stage. By day 5, these parasites proliferate and exhibit high glycolytic score similar to their bloodstream counterparts yet differ in 92 differentially expressed genes (logfc.threshold = 0.25). From days 6 to 7, some parasites differentiate into stumpy forms, which are cell cycle arrested and express stumpy-specific genes. Meanwhile, the slender population remains proliferative, but some start downregulating glycolytic enzymes and flagellum-associated proteins. These results confirm that functional tissue adaptation involves not only a metabolic shift, but it may also affect parasite shape and/or motility. VSG gene expression analysis revealed greater diversity in adipose tissue compared to blood, which is consistent with recent VSG-seq data. In conclusion, our data indicates that adipose-colonizing parasites are primarily slender forms in G1, which can differentiate into stumpy forms or adapt environmentally. We propose that these changes favor the survival of the parasite population, promoting emergence of VSG-switchers and ultimately contributing to disease chronicity.

Session II – Abstracts

Pushing methodology forward

Chair: Joana Faria

Wednesday, September 11

8:45AM – 11:30AM

9 (Plenary) The TrypanoGEN genome-wide association study of susceptibility to trypanosomiasis and insights in the evolutionary trade-off between resistance to infection and susceptivity to non-communicable diseases.

Annette MacLeod, for the TrypanoGEN Research Group, members of The H3Africa Consortium: Julius Mulindwa, Magambo Phillip Kimuda, Harry Noyes, Hamidou Ilboudo, Mathurin Koffi, Bernadin Ahouty, Oscar Nyangiri, Anneli Cooper, Caroline Clucas, Peter Nambala, Walt Adamson, Janelisa Musaya, Dieudonne´ Mumba Ngoyi, Kevin Karume, Olivier Fataki, Gustave Simo, Elvis Ofon, John Enyaru, Barbara Nerima, Andy Tait, Lucio Marcello, John Chisi, Jacques Kabore, Justin Windingoudi Kabore, Kelita Kamoto, Martin Simuunza,Vincent P. Alibu, Vincent Jamonneau, Marianne Camera, Mamadou Camara, Bruno Bucheton, Christiane Hertz-Fowler and Enock Matovu.

Although there has been a steady decline in the number of human trypanosomiasis cases, the disease remains a significant public health concern. The reported cases likely represent only a fraction of the true prevalence, with the growing recognition that asymptomatic carriers serve as hidden human reservoirs, posing a continuous risk of new epidemics. Understanding why some individuals remain asymptomatic while others develop severe disease is crucial to achieving the successful eradication of trypanosomiasis.
To explore the host genetic factors that influence whether an infection remains asymptomatic or progresses to severe clinical outcomes, the TrypanoGEN consortium conducted the first genome-wide association study (GWAS) on human susceptibility to trypanosomiasis. In this talk, I will present the key findings from this study including evolutionary trade-offs, where genetic protection against trypanosomiasis may simultaneously increase the risk of certain non-communicable diseases.

10 New insights into the biology of dermal trypanosomes using a skin-on-chip organoid model

Parul Sharma (Institut Pasteur); Christelle Travaillé (Institut Pasteur); Samy Gobaa (Institut Pasteur); Brice Rotureau (Institut Pasteur)

Trypanosoma brucei is transmitted to the mammalian host by the bite of the tsetse fly vector during a blood meal. The fly deposits infective metacyclic parasites into the skin dermis, from there, the parasites either enter the vascular and lymphatic system or proliferate at the bite site. In the blood, the parasites exist as dividing slender forms and cell cycle-arrested stumpy forms that are adapted to establish infection in the fly during a subsequent blood meal. Experimental infections in animal models and field studies in humans have shown that *T. brucei* maintains a significant population in the extravascular dermis that remain transmissible to tsetse flies. Thus, the skin represents as an important anatomical reservoir for these parasites. To characterise the adaptations of dermal trypanosomes (proliferation, differentiation, transmissibility, motility), we developed an in-house vascularized skin-on-chip (SoC) model within a microfluidic chamber. This skin model including three human cell types shows a tissue organization and a cell polarity mimicking human skin and recapitulating some of its physiological properties. The SoC remains viable for 8 days making it suitable for live and fixed imaging of fluorescent parasites and quantification of key biological functions with high reproducibility. We observed that the parasite motility in the dermal compartment was significantly impacted by the presence of fibroblasts. We also found distinct distribution profiles of stumpy (PAD1+) cells in the dermal compartment as compared to the vascular population, which could be key for parasite transmission.

11 Growth and transcriptomics of micropopulations in semi-permeable capsules to quantify VSG switching frequencies

Anna Barcons-Simon (LMU Munich); Kiryl Kavaliou (LMU Munich); Esteban Osses Soto (LMU Munich); T. Nicolai Siegel (LMU Munich)

Parallel growth of thousands of individual cells to micropopulations followed by transcriptomic analysis can reveal heterogeneity in Variant Surface Glycoprotein (VSG) expression across different trypanosome populations. Although antigenic variation of VSGs is key to immune evasion, quantification of VSG switching frequencies has remained challenging. Plate-based approaches are limited by their inability to support high-throughput growth of micropopulations, which is essential for detecting the low-frequency VSG switching events and thus requires a high number of doubling events analysed. Conversely, droplet-based approaches enable high-throughput single-cell RNA sequencing but are unsuitable for growing micropopulations as their impermeable nature renders cells inaccessible to fresh medium. We have developed a method to grow single trypanosomes contained in semi-permeable capsules (SPCs). In contrast to droplets, the hydrogel shell of our SPCs is porous, allowing the exchange of medium and small molecules while maintaining the compartmentalisation of cells or large biomolecules such as gDNA or RNA. We are currently implementing an approach to sequence the transcriptomes of micropopulations using the SPCs as permeable compartments that keep the cell lysate of the clonal micropopulation together while allowing the diffusion of enzymes and oligonucleotides required for library preparation. This innovative method should enable us to quantify the VSG switching frequencies of slow-switching *T. brucei* lab-adapted lines and fast-switching cow isolates, as trypanosome encapsulation only requires a small portable microfluidics device suitable for field sampling. Additionally, we aim to decipher transcriptomic and genomic features distinguishing slow and fast VSG switching trypanosome populations and other factors contributing to trypanosome cell-to-cell heterogeneity.

12 Molecular tools for functional genetics in *Trypanosoma* vivax

Majeed Bakari-Soale (University of Nottingham); Pieter C Steketee (The Roslin Institute); Rachel Young (The Roslin Institute); Federica Giordani (University of Glasgow); Michael P Barrett (University of Glasgow); Liam Morrison (The Roslin Institute); Bill Wickstead (University of Nottingham); Catarina Gadelha (University of Nottingham)

Trypanosoma vivax is the most widespread trypanosome causing animal African trypanosomiasis (AAT). Despite its huge veterinary importance, it is the least studied among the major species of trypanosomes that cause AAT (*T. brucei*, T. congolense and T. vivax). This is partly due to challenging in vitro cultivation but also to a great dearth of genetic tools for functional studies. The first T. vivax-specific DNA construct was developed over a decade ago for constitutive transgene expression, but since then, there has been little advancement in transgenesis in this parasite. Here we report the development and application of a toolkit for functional genetics using in vitro-differentiated T. vivax trypomastigotes with bloodstream form-like biology. We developed methods to routinely modify these cells at specific loci, and used genomic and transcriptomic data to create species-specific constructs for transgenesis. We identified loci suitable for high-level and tightly regulated expression and generated a line stably expressing T7 RNA polymerase and Tet repressor. Using this, we demonstrate the ability to inducibly silence genes using species-specific RNAi constructs, and the expression of ectopic genes. We further demonstrate gene knockout using conventional knockout and efficient one-step homozygous knockout using Cas9 ribonucleoprotein, and have applied these to investigate the function of genes associated with drug resistance and of relevance to vaccine development. Finally, we developed a new transgenic cell line that produces >10,000 independent clones in a single transfection, providing a potential for generation of genome wide high-complexity mutant libraries. This work brings T. vivax from a point of being essentially untractable, to now being a new model for functional genetics and provides a huge opportunity to address fundamental biological questions in this important but neglected parasite.

13 Establishment of a new system to study in vivo interactions of *Trypanosoma cruzi* and its triatomine insect vector

Ruby E Harrison (University of Georgia CTEGD and Dept of Cellular Biology); Kevin J Vogel (University of Georgia Dept of Entomology); R Drew Etheridge (University of Georgia CTEGD and Dept of Cellular Biology)

Trypanosoma cruzi is the etiological agent of Chagas disease in humans which causes considerable morbidity and mortality in Latin America. *T. cruzi* is a single-celled eukaryote with a dixenous, i.e. two-host, life cycle; parasites alternate between mammals and insect vectors belonging to the subfamily Triatominae (kissing bugs). Research on host-pathogen interactions in this system has primarily focused on stages of *T. cruzi* infecting mammalian hosts while investigation of parasite stages in insects is relatively limited. We established a collaborative research effort among parasitologists and entomologists to characterize *T. cruzi* development in the vector Rhodnius prolixus with improved resolution using molecular methods. Precise quantification of parasite populations in R. prolixus showed that parasites passively move through anterior regions of the insect gut but persist and proliferate in the hindgut (rectum) long-term. We further determined that the clonal and genetically tractable Y strain of *T. cruzi* stably colonizes R. prolixus contrary to prior reports that this strain cannot survive in kissing bugs. Using Y strain *T. cruzi* we launched investigation of parasite gene function in proliferation and life stage transition in triatomine insects using *T. cruzi* mutants generated using CRISPR/Cas genome editing. A sample pilot study showed ablation of cytostome-cytopharynx mediated endocytosis did not adversely affect *T. cruzi* colonization of R. prolixus. In sum, the Y strain *T. cruzi* - R. prolixus system can be leveraged to characterize parasite-insect molecular interactions more powerfully than previously feasible.

14 (TT) Identifying molecular mediators of cellular states in *Trypanosoma cruzi*: comparison of single cell RNA sequencing technologies

Caroline D Keroack (Brown University); Sophie Marcus-Wade (Brown University); Jacqueline Lopez (Brown University); Eric D Salomaki (Brown University); Christopher L de Graffenried (Brown University)

Trypanosoma cruzi differentiates into various cellular states as it progresses through the insect host, shifting from the proliferative epimastigote to the infectious metacyclic trypomastigote. The cell cycle of chemically synchronized epimastigotes and the expression profiles of purified metacyclic trypomastigotes have been described using bulk RNA sequencing. These methods of synchronization or purification can be harsh to the cell and may alter gene expression. Single-cell RNA -sequencing is an ideal method for profiling the cell cycle and metacyclogenesis, as both are asynchronous in culture. Here, we demonstrate that *T. cruzi* polyadenylates ribosomal RNA, resulting in significant rRNA contamination in data generated using the 10X genomics pipeline, which relies on oligo(dT) capture. Deep sequencing can be employed to capture full transcriptomes from these samples, but this represents a significant financial burden and impacts the data quality. To address these issues, we have compared two scRNA-seq technologies – the microfluidic based 10X genomics platform and particle-templated instant partition sequencing (PIPseqTM, Fluent Biosciences) – to assess the relative rRNA contamination and compare the cluster markers identified. We show that PIPseq results in significantly fewer reads mapping to rRNA, resulting in higher coverage of transcripts of interest. Using these data, we have identified both conserved and *T. cruzi*-specific markers of cell cycle progression in *T. cruzi* epimastigotes. We also discovered marker genes of metacyclic trypomastigotes which further demonstrate significant reprogramming during metacyclogenesis. We are now focusing on using immunofluorescence microscopy and live cell imaging to validate candidate genes important to the different cellular states.

15 (TT) LeishGEM: A genome-scale database for knockout mutant life cycle fitness phenotyping and subcellular protein localisation in *Leishmania mexicana*

Ulrich Dobramysl (University of Oxford); Eden R Ferreira (University of York); Rachel P Neish (University of York); Laura D Davidson (Oxford Brookes University); Raquel Pereira (University of Bern); Ruth Etzensperger (University of Bern); Sidonie Aellig (University of Bern); Matthew Young (University of Glasgow); James Smith (University of Glasgow); Jeziel Damasceno (University of Glasgow); Jack D Sunter (Oxford Brookes University); Jeremy Mottram (University of York); Eva Gluenz (University of Bern); Richard Wheeler (University of Oxford)

The success of *Leishmania* parasites as pathogens is encoded in their genome. However, as eukaryotes, their genome is large and relatively complex. Despite extensive efforts for the functional characterisation of protein-coding genes in *Leishmania*, the role and localisation of most is still unclear. According to TriTrypDB only 14% of the 8,267 *L. mexicana* protein-coding genes have been unambiguously named, with the large majority remaining of putative function or hypothetical. The major aims of the *Leishmania* Genetic Modification (LeishGEM) project are to systematically address this by: 1) Determining the fitness of deletion mutants of protein-coding genes (genome-wide, 8,267 genes) by generating uniquely genetically barcoded deletion cell lines and assessing growth fitness as promastigotes, axenic amastigotes, amastigotes in macrophages, and in a mouse footpad infection. 2) Visualising the sub-cellular localisation of proteins in promastigotes and axenic amastigotes by tagging at both the N and C termini (if lacking an ortholog in or divergent from *T. brucei* - 2,700 target genes) in the LeishTag sub-project. 3) Analysing protein subcellular localisation via LOPIT-DC fractionation. The fitness phenotyping and localisation data generated by the LeishGEM project is available as we generate it at https://browse.leishgem.org/. As of June 2024, this database contains fitness data for 2,305 gene deletion mutants, subcellular localisation for 1,426 tagged cell lines where we have completed localisation annotation, and 3,782 proteins for which LOPIT-DC fractionation and mass spectroscopy yielded information on the subcellular localisation. This is the first data release of a transformative resource for the function of thousands of genes in a family of important human pathogens.

16 (TT) A novel conditional knockdown system in *T. cruzi* reveals essential roles for Aurora and Polo-like kinases in parasite cell division

Justin Wiedeman (University of Georgia); Ruby Harrison (University of Georgia); Drew Etheridge (University of Georgia)

The protozoan parasite *Trypanosoma cruzi* is responsible for Chagas disease, the most severe parasitic illness of the Americas. Despite the medical importance of *T. cruzi*, our knowledge of the identity and function of essential genes is severely limited by the lack of a conditional knockdown system. Unlike its more genetically tractable cousin *Trypanosoma brucei*, *T. cruzi* lacks the necessary enzymatic machinery to facilitate RNAi based functional studies. Additionally, the diploid nature of the *T. cruzi* genome and arrangement of genes into polycistronic "cassettes" which lack clearly defined promoters, precludes the use of standard molecular approaches to analyze essential gene function. Here we describe the development and use of a functional conditional knockdown system based on a tetracycline-responsive hammerhead ribozyme (HHR) inserted into the 3' un-translated (UTR) region of genes of interest. Using this new tool, we demonstrated the essential nature of multiple protein-coding genes, including the genes for the Aurora and Polo-like kinases. We discovered roles for these kinases in mitosis and cytokinesis, respectively, with these studies mirroring their known roles in *T. brucei*. Importantly, we demonstrated the effectiveness of the HHR knockdown system in intracellular amastigotes, the medically relevant mammalian stage of the parasite. This conditional knockdown system allows, for the first time, the straightforward functional characterization of essential genes in *T. cruzi*.

17 (TT) A 2A peptide-based epitope-tagging toolkit for analysis of protein localisation, regulation, and function in Trypanosomatids

Carla Gilabert Carbajo (Imperial College London); Xiaoyang Han (Imperial College London); Bhairavi Savur (Imperial College London); Arushi Upadhyaya (Imperial College London); Fatima Taha (Imperial College London); Richard J Wheeler (University of Oxford); Michele Tinti (University of Dundee); Phillip Yates (Oregon Health and Science University); Calvin Tiengwe (Imperial College London)

Kinetoplastid parasites cause serious diseases affecting human and animal health. As these parasites transition between vertebrate hosts and insect vectors, their survival relies on precise gene expression regulation to adapt to environmental changes such as temperature and nutrient availability. Since gene regulation in Kinetoplastids is primarily post-transcriptional, developing an efficient gene tagging tool that retains important cis-regulatory elements is crucial for investigating protein localisation and function under specific conditions. Here, we present a new tagging system that preserves cis-acting gene regulatory elements. Our system combines CRISPR/Cas9-mediated editing with the 2A peptide-based system to achieve bicistronic expression of a drug-selectable marker and a tagged gene from its endogenous locus. The 2A peptide sequence causes a “ribosomal skip” event during translation, allowing the single bicistronic transcript to produce separate functional proteins. We demonstrate the functionality of our system by tagging six *Trypanosoma brucei* proteins with mNeonGreen or mScarlet in different cellular compartments: ESAG3 (ER), cytosolic Hsp70, a subunit of the iron transporter the transferrin receptor (ESAG7), two cytoskeletal calpain-related proteins (CAP5.5, CAP5.5V), and glycosylphosphatidylinositol phospholipase C (GPI-PLC). We show: (i) high-efficiency separation of drug marker and target 2A-modified protein modified, (ii) >90% positive selection of all transgenic cell lines, (iii) differential regulation of Hsp70 to heat shock, (iv) iron-dependent regulation of ESAG7 and functionality by transferrin endocytosis, and (v) stage-specific developmental regulation of CAP5.5, CAP5.5V, and GPI-PLC during in vitro differentiation. Our 2A-based tagging toolkit is applicable to all kinetoplastids amenable to CRISPR/Cas9 gene editing. It is useful for studying post-transcriptional and post-translational regulation within a single experiment.

Session III – Abstracts

Cell biology

Chair: Joseph Smith

Wednesday, September 11

2:00PM – 4:45PM

18 *Leishmania mexicana* flagellum attachment zone is required for adhesion to substrates

Barrack O Owino (Oxford Brookes University); Ryuji Yanase (University of Leicester); Alan Marron (Oxford Brookes University); Flavia Moreira-Leite (Oxford Brookes University); Sue Vaughan (Oxford Brookes University); Jack D Sunter (Oxford Brookes University)

Leishmania has multiple developmental stages in the sand fly, including the understudied haptomonad, which is important for enhancing transmission and is attached to the sand fly stomodeal valve using its highly modified flagellum. Adhesion is maintained by a complex attachment plaque from which filaments extend towards the cell body, which likely connects into the flagellum attachment zone (FAZ), a large cytoskeletal structure important for cell morphogenesis. We had previously identified three essential proteins for haptomonad adhesion: kinetoplastid-insect adhesion proteins 1-3 (KIAPs1-3); yet, we know little about the role of the FAZ in adhesion. Here, we generated FAZ2, FAZ5, and FAZ34 deletion mutants and used a combination of light and electron microscopy to examine their roles in *Leishmania* adhesion. Interestingly, deletion of any of these FAZ proteins impaired haptomonad adhesion in vitro, with FAZ34 loss causing a mispositioning of the haptomonad cell body and a reduction in KIAP1 and KIAP3 in the attachment plaque. Electron micrographs of whole-mount cytoskeletons showed that the positioning of a novel set of filaments in the flagellum associated with the anterior cell tip was disrupted in the FAZ34 deletion mutant. Moreover, immunoelectron microscopy of parental and KIAP2 variant expressing a myc epitope showed that KIAP2 was a fundamental component of these flagellar filaments. Overall, we show the importance of the FAZ for parasite adhesion to substrates, indicating that a strong connection to the cell body is critical for stable adhesion.

19 A novel approach to tagging tubulin reveals microtubule assembly dynamics of the flagellum in *Trypanosoma brucei*

Daniel Abbühl (Institut Pasteur Paris); Serge Bonnefoy (Institut Pasteur Paris); Bastin Philippe (Institut Pasteur Paris)

During its complex life cycle, *Trypanosoma brucei* progresses through multiple stages that all have an intricate flagellum length. Here we followed flagellum assembly and length control with the flagellum's main component, tubulin. By employing a novel intragenic tagging strategy for tubulin, we obtained for the first time, a protein behaving indistinguishable from its untagged counterpart. After inducible expression of Ty-1-tubulin, we dissected microtubule assembly dynamics inside the flagellum during the cell cycle. Furthermore, we managed to trace the fate of individual flagella several cell cycles after their emergence. These experiments delivered direct evidence how procyclic trypanosomes manage to assemble a new flagellum while maintaining an existing one in the same cell: new tubulin is only incorporated into the growing flagellum at a linear rate but not the old flagellum while cells are bi-flagellated. This supports a locking mechanism at the base of the mature flagellum, established by the CEP164C protein (Atkins et al. 2021) to prevent competition between the two flagella. We formally demonstrated this by showing that depletion of CEP164C leads to simultaneous integration of tubulin in both flagella. However, elongation was more extensive in the new flagellum, indicating the lock is not the only regulator. Furthermore, after the cell divides one cell inherits the new and one the old flagellum that are now no longer competing. The previously old flagellum gets unlocked briefly and incorporates new material at the distal tip. This suggests regular turn-over, as frequent integration during G1-phase would lead to a net elongation over time.

20 Flagellar distal-end proteome is critical for axonemal construction and length regulation in *Trypanosoma brucei*

Hana Pavliskova (Institute of Molecular Genetics of the Czech Academy of Sciences); Peter Gorilak (Institute of Molecular Genetics of the Czech Academy of Sciences); Ludek Stepanek (Institute of Molecular Genetics of the Czech Academy of Sciences); Marie Zelena (Institute of Molecular Genetics of the Czech Academy of Sciences); Bill Wickstead (University of Nottingham); Vladimir Varga (Institute of Molecular Genetics of the Czech Academy of Sciences)

The flagellum of *Trypanosoma brucei* is essential for trypanosome cell motility, morphogenesis and signalling. The core cytoskeletal structure of the flagellum is the microtubule-based axoneme, which is complex, highly organized, and well conserved across eukaryotes. The axoneme is constructed by addition of material to its distal end, with the axonemal length being tightly regulated. How are these processes orchestrated remains poorly understood. Validating data of the *T. brucei* genome-wide protein localization resource TrypTag.org yielded nearly 80 proteins enriched at the distal end of its flagellum. This set comprises both kinetoplastid specific and evolutionarily conserved proteins, including members of families regulating microtubule lengths, such as CLASPs, kinesin-13s, and katanins. We performed a comprehensive characterization of the distal end proteins including description of their depletion phenotypes. This revealed a group recruited to the distal end prior to the flagellum reaching its full length, which is responsible for limiting the axonemal growth. Other proteins, present exclusively at the end of a growing flagellum, contribute to the axonemal construction in a protein-specific manner, such as by facilitating a synchronized growth of axonemal microtubules. Some members of this group belong to evolutionarily conserved yet functionally uncharacterized families and we showed that their mammalian orthologs also localize to the distal end of mammalian cilia. This work reveals the importance of the distal end proteins for the regulation of axonemal growth and thereby morphogenesis of *T. brucei*. Moreover, it represents the first comprehensive analysis of the flagellum distal-end proteome in a eukaryote, identifying evolutionarily conserved features.

21 Tubulin detyrosination shapes *Leishmania* cytoskeletal architecture and virulence

Rosa Milagros Corrales (University of Montpellier, CNRS, IRD, MiVEGEC); Jeremy Vincent (University of Montpellier, CNRS, IRD, MiVEGEC); Lucien Crobu (University of Montpellier, CNRS, IRD, MiVEGEC); Rachel Neish (York Biomedical Research Institute and Department of Biology, University of York); Binita Nepal (Department of Pediatrics and Department of Biochemistry, University of Texas Southwestern Medical Center); Julien Espeut (Tubulin Code team, IGH, CNRS); Grégoire Pasquier (University of Montpellier, CNRS, IRD, MiVEGEC); Ghislain Gillard (Tubulin Code team, IGH, CNRS); Chantal Cazevieille (Université Montpellier, INSERM U1298, INM); Jeremy Mottram (York Biomedical Research Institute and Department of Biology, University of York); Dawn Wetzel (Department of Pediatrics and Department of Biochemistry, University of Texas Southwestern Medical Center); Yvon Sterkers (University of Montpellier, CNRS, IRD, MiVEGEC); Krzysztof Rogowski (Tubulin Code team, IGH, CNRS); Maude F Leveque (University of Montpellier, CNRS, IRD, MiVEGEC)

Tubulin detyrosination has been implicated in various human disorders and is important for regulating microtubule dynamics. While in most organisms this modification is restricted to α-tubulin, in Trypanosomatid parasites, it occurs on both α- and β-tubulin. Here we show that, in *Leishmania*, a single vasohibin (LmVASH) enzyme is responsible for differential kinetics of detyrosination. LmVASH knockout parasites are completely devoid of detyrosination and show decreased levels of tubulin glutamylation. Loss of detyrosination alters microtubule dynamics resulting in impaired developmental morphogenesis. Detyrosination-deficient amastigotes display altered cell division, morphology and flagellum remodeling that was associated with diminished proliferation in macrophages and pathogenicity in mice. We show that the microtubule depolymerizing Kinesin-13.2 is a functionally relevant reader of the “tubulin code” required for flagellum shortening. Taken together, our work establishes the importance of tubulin detyrosination in remodeling the microtubule-based cytoskeleton that is required for efficient infection and proliferation in the mammalian host.

22 How to attach the TAC

Salome Aeschlimann (DCBP, University of Bern); Caroline Dewar (University of Lancaster); Clirim Jetishi (ICB, University of Bern); Bianca Berger (ICB, University of Bern); Bernd Schimanski (DCBP, University of Bern); Philip Stettler (DCBP, University of Bern); Silke Oljeklaus (Biozentrum, University of Würzburg); Bettina Warscheid (Biozentrum, University of Würzburg); Torsten Ochsenreiter (ICB, University of Bern); André Schneider (DCBP, University of Bern)

The tripartite attachment complex (TAC) physically connects the mitochondrial genome of *T. brucei* to the basal body (BB) and ensures correct segregation of the mitochondrial genome during the cell cycle. We were able to show that the BB most proximal TAC subunit p197, a large protein (> 600 kDa), spans the entire cytosolic part from BB and the pro-BB to the mitochondrial outer membrane. How the connection of the TAC to the BB is maintained is currently unknown. Here we provide evidence for a specific interaction of p197 and the trypanosome specific BB protein KMP11. In a quantitative pulldown experiment with KMP11 as the bait, p197 was highly enriched. We further confirmed this putative interaction using pulldown experiments under stringent lysis conditions and show that the C-terminus of p197 interacts with KMP11. Additionally, depletion of KMP11 results in a minor delocalization of p197 from the newly formed pro-BBs. p197 functions as a flexible spacer spanning over 270 nm, with predicted large alpha-helical stretches forming flexible coiled-coil domains. This possibly allows the protein to assume various lengths. Surprisingly, ultrastructure expansion microscopy revealed KMP11's localization along the entire length of p197. This suggests that KMP11 additionally helps to stabilize p197 filaments and aides their flexible folding. Thus, KMP11 might not only attach the TAC to the BB but also provide stabilization in its flexibility.

23 Better together: the importance of ATP synthase dimerization for cristae maturation and OXPHOS

Michaela Husová (Biology Centre, Institute of Parasitology; The University of South Bohemia, Faculty of Science); Martin Zoltner (School of Life Sciences, University of Dundee; Department of Parasitology, Faculty of Science, BIOCEV, Charles University); Mark Field (Biology Centre, Institute of Parasitology; School of Life Sciences, University of Dundee); Alena Zíková (Biology Centre, Institute of Parasitology; The University of South Bohemia, Faculty of Science)

To efficiently infect the insect vector, the long slender bloodstream form (LS-BF) of *Trypanosoma brucei* differentiates into the short stumpy form (SS-BF). Both forms exhibit reduced mitochondria with almost no cristae and no capacity for oxidative phosphorylation (OXPHOS), though SS-BF has a larger mitochondrial proteome. During the transition to the procyclic form (PF), discoidal cristae appear in 12 hours, and electron transport chain complexes III and IV in 24 hours. Fully differentiated PF uses OXPHOS for ATP generation by ATP synthase, a multifaceted complex that is also involved in shaping discoidal cristae by forming dimers. Studying the ATP synthase´s role in cristae biogenesis is challenging due to its primary function as an OXPHOS enzyme, but the SS-BF to PF transition offers a unique opportunity to investigate this phenomenon. LS-BF double knock-out (DKO) parasites lacking the ATP synthase subunit g possess only the monomeric, fully functional enzyme. They can differentiate into SS-BF but exhibit lower parasitemia in vivo and die within six hours of differentiating into PF, coinciding with cristae maturation but before OXPHOS is established. After several weeks in culture, LS-BF DKO parasites can surprisingly differentiate into PF. These mutants replicate like wild-type PF but have altered mitochondrial structure, reduced respiration, and significantly lower mitochondrial ATP production from amino acid oxidation. Glucose is toxic to them, suggesting reliance on another energy source, possibly fatty acids. This study highlights the critical role of ATP synthase dimers in cristae maturation and the extreme metabolic adaptability of trypanosomes to laboratory settings.

24 Functional characterization of compartmentalized cAMP signals in *Trypanosoma cruzi*

Milad Ahmed (University of Cincinnati); Miguel Chiurillo (University of Cincinnati); Noelia Lander (University of Cincinnati)

Trypanosoma cruzi, the causative agent of Chagas disease, differentiates into four developmental stages during its digenetic life cycle. These transformations are regulated by signaling pathways, among which cAMP has been shown to mediate cell adhesion, metacyclogenesis, and osmoregulation in *T. cruzi*. However, this signaling cascade remains largely unexplored in trypanosomes. In mammalian cells, cAMP signals are spatiotemporal regulated by compartmentalization in subcellular microdomains that are maintained by phosphodiesterases (PDEs), enzymes that hydrolyze cAMP into AMP. Our laboratory recently identified two putative cAMP microdomains in *T. cruzi*: the flagellar tip and the contractile vacuole complex (CVC), but their specific role remains elusive. We hypothesize that *T. cruzi* PDEs play a role in cAMP compartmentalization and function by limiting its diffusion near the synthesis sites. Previous research found that TcPDEB1 and TcPDEB2 localize to the flagellum, while TcPDEC is present in the CVC. Taking advantage of this differential localization, we aimed to characterize these two compartments as cAMP microdomains in *T. cruzi*. We confirmed the localization of TcPDEs by CRISPR/Cas9-mediated endogenous gene tagging. Subsequently, we generated PDEs overexpression, knockout, and addback cell lines to modulate the content of cAMP in each domain and investigate their individual role. We found that cAMP from the CVC regulates cell volume recovery in response to hypoosmotic stress, while flagellar cAMP mediates the differentiation of vector forms (metacyclogenesis), and mammalian host cell invasion in *T. cruzi*. Our results revealed clearly distinguished functions for these two signaling microdomains, highlighting the importance of cAMP compartmentalization in *T. cruzi*.

25 (TT) The hydrophobic effect drives the outer-to-inner mitochondrial membrane connection of the Trypanosomal tripartite attachment complex

Philip Stettler (University of Bern); Salome Aeschlimann (University of Bern); Bernd Schimanski (University of Bern); Sandro Käser (University of Bern); André Schneider (University of Bern)

Genome inheritance is essential for all lifeforms. Trypanosomes are single celled organisms harboring a single mitochondrion that use a unique protein complex, the tripartite attachment complex (TAC), to segregate their mitochondrial DNA. The TAC links the mitochondrial DNA network to the flagellum, ensuring its proper segregation during cytokinesis. This study focuses on a key connection within the TAC between two of its subunits: TAC60 and p166. TAC60 is a protein of the outer mitochondrial membrane and interacts with p166, a membrane protein of the inner mitochondrial membrane. This interaction creates a unique mitochondrial membrane contact site. Here, we have investigated this interaction using an AlphaFold structure model as well as an in vitro peptide-protein interaction screen. This resulted in the discovery of a well conserved kinked α-helix in TAC60 at the binding site of p166. In vivo experiments revealed that the tertiary structure of this α-helix is crucial for its interaction with p166. Surprisingly, some highly conserved charged amino acids are dispensable for this interaction. Instead, less well conserved hydrophobic amino acids in the interaction motifs of both TAC60 and p166 mediate the interaction which is therefor a result of the 'hydrophobic effect'. Consequently, the protein interaction is very stable in vivo and displays a high tolerance to increased salt concentrations in vitro. In summary, we characterized the central interaction connecting the cytosolic and the inner module of the Trypanosomal TAC. This is the first study to investigate the integrity of the TAC on a sub-protein level.

26 (TT) Bicarbonate – CO2 sensing and response mediated by trypanosome QIQ1

Anna Trenaman (University of Dundee); Federico Rojas (University of Edinburgh); Michele Tinti (University of Dundee); Keith Matthews (University of Edinburgh); Sam Alsford (London School of Hygiene and Tropical Medicine); David Horn (University of Dundee)

African trypanosomes occupy distinct environments in their mammalian host, and during their life cycle, and adapt their metabolism accordingly. A quorum-sensing pathway has been described in bloodstream-form trypanosomes, for example, involving oligopeptide-sensing and differentiation, but the pathways involved in sensing and responding to other environmental cues remain incompletely characterised. We now describe a bicarbonate-sensing mechanism. A genome-scale loss-of-function genetic screen revealed knockdowns associated with gain-of-fitness, those that increase trypanosome growth. The dominant hit (Tb927.8.6870) encodes a flagellum-localised protein with multiple putative calmodulin binding IQ-domains. We name this protein QIQ1, for Quintuple IQ-domain protein 1, also reflecting the 'quick' growth phenotype observed following knockdown. qiq1-null trypanosomes display a growth advantage both in vitro and in vivo; in culture and in a mouse model, respectively. These mutants maintained morphological differentiation and expression of a quorum-sensing marker in vivo indicating placement of QIQ1 in a distinct pathway. The IQ domains suggested a calcium-signalling mechanism, and consistent with this view, calcium chelators abolished the competitive advantage displayed by qiq1-null cells. We tested several potential environmental cues and found that the competitive advantage of qiq1-null cells was abolished in the absence of sodium bicarbonate. Proteomic analysis revealed a deficit in the modulation of mitochondrial ATP-synthase and citric acid cycle enzymes in the absence of QIQ1, while preliminary metabolomic analysis revealed specific shifts in ATP levels, and in other nucleotides and amino acids, consistent with metabolic remodelling. We conclude that trypanosomes sense bicarbonate in a QIQ1- and calcium-dependent manner and elicit a metabolic response. This response to an external stimulus may facilitate adaptation to resource-limiting environments.

27 (TT) A genome-wide overexpression screen identifies proteins that inhibits the growth of *Trypanosoma brucei*

Navina Panneer Selvan (Rutgers University); Aditi Mukherjee (Rutgers University); Esteban Erben (IIBIO-UNSAM); Hee-Sook Kim (Rutgers University)

Trypanosoma brucei is a protozoan parasite that causes African trypanosomiasis in humans and animals in sub-Saharan Africa. The disease is fatal if left untreated and proceeds from a peripheral to a central nervous system (CNS) infection. Development of vaccines have been hampered due to the surface antigen switching occurring in *T. brucei* cells, which allows the parasite to escape the host immune recognition. Genome-wide screening tools can facilitate the identification of potential drug target proteins and pathways. Gene depletion method using RNAi library has been used for such studies in *T. brucei*. To develop a complementary gain-of-function tool, we generated *T. brucei* overexpression (OE) libraries and validated them in drug resistance screening (Carter et al, 2020), which discovered proteins that confer resistance to melarsoprol (a drug for stage II HAT). Overexpression of some genes can inhibit cell proliferation, while deletion of them has no effect. To identify 'toxic' genes that inhibit the growth of *T. brucei* cells when they are overexpressed, we screened ~400,000 OE library transfected trypanosome cells and isolated about 200 'toxic' genes using a targeted high-throughput DNA-seq. We validated three candidates (HYP1, HYP2, and HYP3) for *T. brucei* cell growth inhibition. Through RNA-seq analysis, we discovered that HYP1 and HYP2 are involved in transcriptional silencing and HYP3 is important for cell-cycle regulation. We will discuss how the overexpression of these proteins might affect the growth of *T. brucei*.

28 (TT) Host-pathogen dynamics of bumble bee parasite, *Crithidia bombi*

Blyssalyn V Bieber (Villanova University); Faith St. Clair (Villanova University); Sarah Lockett (Villanova University); Sonja Glasser (University of Massachusetts Amherst); Lynn S Adler (University of Massachusetts Amherst); Megan L Povelones (Villanova University)

Crithidia bombi is a monoxenous Trypanosomatid that infects bumble bees by attaching to their intestinal tract, impacting survival of workers and overwintering queens. There is extensive research on the ecology of this host-pathogen system, however, less is known about the cellular mechanisms mediating infection dynamics. Researchers typically maintain *C. bombi* in laboratory-reared bumble bee (Bombus impatiens) colonies, but parasites can also be culture adapted. We obtained 30 clonal isolates from laboratory B. impatiens and are examining their phenotypic and molecular diversity, including sequencing to determine haplotypes. This data will be compared to other field isolates providing a baseline for studies on host specificity and determinants of infection. We modified one isolate to express either GFP or RFP and used these parasites to infect B. impatiens, allowing for in vivo visualization. By expanding the genetic toolkit for *C. bombi*, we will better understand factors that affect parasite survival and host colonization. For example, sunflower (Helianthus annuus) pollen has been shown to prevent and reduce *C. bombi* infections in bumble bees. We explored this interaction in vitro by applying sunflower pollen to cultured *C. bombi* parasites expressing a nano-Luciferase construct. While growth was unaffected by pollen exines derived from either buckwheat or sunflower, sunflower pollen specifically disrupted attachment of parasites to tissue culture plates. Furthermore, sunflower exines damage or remove attached *C. bombi* growing as rosettes. We are continuing to explore this interaction to better understand how sunflower exines might act on parasites in vivo to reduce pathogen transmission in natural settings.

Session IV – Abstracts

Genomes and their architecture

Chair: Gaelle Lentini

Thursday, September 12

8:45AM – 11:30AM

29 (Plenary) Mechanisms of antigen diversification in *Trypanosoma brucei*

Monica Mugnier (Johns Hopkins School of Public Health)

Trypanosoma brucei employs a sophisticated mechanism of antigenic variation to evade recognition by the host immune system. Coated in a dense variant surface glycoprotein (VSG) coat that effectively obscures immune recognition of other, invariant, proteins on the parasite's surface, the parasite periodically "switches" its VSG to evade host antibody. Multiple line of evidence suggest, however, that the repertoire of VSGs encoded in the parasite's genome may not be large enough to sustain immune evasion through VSG switching alone. To account for this antigenic shortage, *T. brucei* diversifies, and thus extends, its VSG repertoire through the generation of "mosaic" VSGs, novel variants formed through recombination. Though mosaic VSGs are critical for maintaining infection, the mechanisms driving their formation remain poorly understood, primarily due to a lack of tractable experimental tools for studying these novel antigens. To understand the mechanisms driving mosaic VSG formation, we have developed a highly sensitive targeted sequencing approach for measuring VSG diversification. Using this method, we show that a Cas9-induced DNA double-strand break within the VSG coding sequence can induce VSG recombination with patterns identical to those observed during infection. Our analysis of thousands of mosaic VSG recombination events reveals patterns consistent with specific DNA repair mechanisms, as well as a possible hypervariable region within the VSG. Together, these results provide insight into the mechanisms of VSG diversification and an experimental framework for studying the evolution of antigen repertoires in pathogenic microbes.

30 Nanopore sequencing-based deep learning assay reveals the complete DNA replication landscape in *Leishmania* and its connection with genome variability.

Jeziel D. Damasceno (University of Glasgow); Gabriel L.A. da Silva (University of Glasgow); Catarina A. Marques (University of Glasgow); Craig Lapsley (University of Glasgow); Dario Beraldi (University of Glasgow); Richard McCulloch (University of Glasgow)

Genomic plasticity, driven by gene and chromosome copy number variation, is a pivotal adaptive mechanism in *Leishmania*, including during drug resistance evolution. The relationship between such genomic flexibility and the mechanisms of genome maintenance and duplication, however, remains poorly understood. Here, we employed D-NAscent, a deep learning-based assay utilizing Oxford Nanopore sequencing, which not only confirmed the dominance of a single major locus of DNA replication initiation in each chromosome, as we have previously shown, but also uncovered thousands of previously undetected, highly stochastic replication initiation events. We show that larger chromosomes exhibit a higher density of stochastic initiation events compared to their smaller counterparts, suggesting an evolutionary adaptation to mitigate their delayed replication timing. Additionally, genome-wide analysis of Origin Efficiency Metrics indicated that stochastic initiation zones are characterized by high AT content, elevated G-quadruplex (G4) levels, reduced chromatin occupancy, and decreased transcription initiation activity. Further, we show that DNA replication initiation efficiency is associated with increased mutagenesis, as demonstrated by the accumulation of single nucleotide polymorphisms (SNPs). Collectively, our findings provide a comprehensive overview of the DNA replication landscape in *Leishmania*, illustrating that genome duplication is facilitated by a single, likely constitutive origin in each chromosome, supported by more widespread, potentially stochastic replication events whose distribution reflects chromosome size and dictate replication timing and genomic variability. These insights offer a deeper understanding of *Leishmania* genome malleability and adaptability.

31 Structural characterisation of nucleosomes from *Trypanosoma brucei* reveals unique features of the Trypanosomatid chromatin landscape

Gauri Deák (University of Edinburgh); Hannah Wapenaar (University of Edinburgh); Gorka Sandoval (University of Edinburgh); Hayden Burdett (University of Edinburgh); Ruofan Chen (University of Edinburgh); Mark Taylor (University of Edinburgh); James Watson (University of Edinburgh); Maarten Tuijtel (University of Edinburgh); Martin Singleton (University of Edinburgh); Shaun Webb (University of Edinburgh); Marcus Wilson (University of Edinburgh)

Trypanosoma brucei is a kinetoplastid that causes severe disease in both humans and livestock animals. Contrary to model eukaryotes, Trypanosomatid histones are highly divergent. In *T. brucei*, histone variants serve as architectural regulators of transcription and monoallelic expression of variant surface glycoprotein genes. However, the structural and functional consequences of histone sequence variation on these chromatin processes are unknown. We determined the structures of both canonical and variant nucleosomes from *T. brucei* using single particle cryo-EM and leveraged in vitro biochemistry to identify alterations in the way trypanosome histones assemble into nucleosomes. We found that canonical nucleosomes in *T. brucei* are unstable and exhibit weakened DNA binding at nucleosome entry/exit sites. Our structure shows that this is compensated by a novel, Trypanosomatid-specific mechanism at the H2A-H2B dimer interface. Surprisingly, the acidic patch, a well-characterized protein interaction hotspot in other organisms, is highly modified in *T. brucei* and refractory to known binders. We see that the incorporation of histone variants further alters the DNA binding and protein interaction potential of *T. brucei* nucleosomes. The repressive histone variant H3.V that regulates antigenic variation and defines transcription termination regions leads to an unexpected splaying of nucleosomal DNA ends. The activating variants H2A.Z and H2B.V that define transcription start regions substantially alter the acidic patch, suggesting that critical interactions with chromatin factors may be fine-tuned to control transcription. Overall, our findings shed light on the molecular complementarity between histone evolution and chromatin biology in trypanosomes and may open new therapeutic avenues.

32 Comparative analysis of canonical and non-canonical nuclear genetic codes in Trypanosomatids

Kristina Pasutova (Institute of Parasitology); Zoltan Fussy (Scripps Institution of Oceanography); Amanda Albanaz (University of Ostrava); Jan Votypka (Institute of Prasitology); Anzhelika Butenko (Institute of Parasitology); Ambar Kachale (Institute of Parasitology); Alexei Kostigov (University of Ostrava); Vyacheslav Yurchenko (University of Ostrava); Julius Lukes (Institute of Parasitology)

Recently, a departure from the canonical genetic code was described in *Blastocrithidia* nonstop. This monoxenous Trypanosomatid reassigned all three stop codons to code for amino acids (UAA and UAG for glutamate, UGA for tryptophan), with UAA having dual meaning, as it also specifies translation termination. While UAA and UAG are decoded by cognate tRNAs that perfectly match the anticodon, UGA is recognized by a specific tRNATrpCCA with its anticodon stem uniquely shortened from five to four base pairs, allowing readthrough over this stop codon. We wondered whether this genetic code reassignment is confined to B. nonstop or is a hallmark of a larger group of Trypanosomatids. To answer this question, we sequenced the genomes of three other *Blastocrithidia* species and four Obscuromonas species that represent a sister lineage to *Blastocrithidia* and utilize a canonical genetic code. The results show that the common ancestor of *Blastocrithidia spp.* already had a GC-poor genome with all three stop codons reassigned and only UAA employed as a genuine stop. Although the specific mechanism how the translation is terminated remains unknown, remarkably, in *Blastocrithidia* species UAA is significantly enriched after the genuine stop, while no such distribution was observed in Obscuromonas species. We have identified numerous protein-coding genes that were either lost or gained specifically at the *Blastocrithidia* node and are likely incompatible with or essential for the altered code, respectively. The across-species comparison of the in-frame stop codons revealed their non-random distribution, with abundant proteins being remarkably stop codon-poor. This comparative analysis allows the identification of numerous features associated with the reassigned nuclear genetic code.

33 Chromatin three-dimensional organization into compartments and domains in *Trypanosoma cruzi*

Florencia Díaz-Viraqué (Institut Pasteur Montevideo); María L Chiribao (Institut Pasteur de Montevideo; UdelaR); Gabriela Libisch (Institut Pasteur Montevideo); Carlos Robello (Institut Pasteur de Montevideo; UdelaR)

Gene organization, regulation of genome expression, and RNA metabolism in trypanosomes differ from that in other eukaryotes. It has been widely accepted that post-transcriptional regulation is the main mode of gene expression regulation in *Trypanosoma cruzi*, the causative agent of Chagas disease. The genome of *T. cruzi* is partitioned into two large regions named compartments: the core (highly conserved and syntenic among Trypanosomatids) and the species-specific disruptive compartment (synteny disruption) containing multigene families encoding for surface proteins. By mapping genome-wide chromatin interactions using chromosome conformation capture (Hi-C) data, we demonstrated that the genome is organized into three-dimensional (3D) compartments and domains. In particular, we showed that core and disruptive regions of the *T. cruzi* genome constitute 3D compartments that we named C and D, analogous to A and B compartments in other eukaryotes. We observed that these compartments present an asymmetrical distribution of DNA methylation marks, nucleosome positioning, and chromatin interactions, affecting genome expression dynamics. Besides, the expression is influenced by local chromatin structure through the organization of the genome into chromatin folding domains (CFD). Our results support a new model in which epigenetic mechanisms dramatically impact gene expression in this eukaryotic pathogen. We also extended our analyses to the related parasite *Trypanosoma brucei* and concluded that these findings are common to both organisms.

34 Hidden origami in *Trypanosoma cruzi* nuclei highlights its nonrandom 3D genomic organization

Natália Karla Bellini (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil); Pedro Leonardo Carvalho de Lima (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil); David da Silva Pires (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil); Julia Pinheiro Chagas da Cunha (Cell Cycle Laboratory, Butantan Institute, São Paulo, Brazil; Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, Brazil)

The protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, exhibits polycistronic transcription and unidimensional genome compartmentalization of core (conserved) and disruptive (virulence factors from multigenic families) genes, with approximately 50% of its genome being repetitive, mainly comprising virulence factor genes. Genomic sequences, particularly those associated with repeats, motifs of architectural proteins, and noncoding RNA loci, are crucial for genome folding. Here, we evaluated the genomic features associated with higher-order chromatin organization in *T. cruzi*. We conducted extensive computational processing of high-throughput chromosome conformation capture (Hi-C) data, accounting for repetitive regions and improvements in genome annotation. Our study revealed that repetitive DNA (multimapped reads) significantly influences 3D chromatin folding, particularly in determining the TAD boundaries. In contrast to core genes, virulence factor genes form shorter and more compact TAD-like domains enriched in loops, suggesting a gene expression regulatory mechanism. We found a nonrandom 3D organization of the genome, where nonprotein-coding RNA loci (e.g., tRNAs, snRNAs, and snoRNAs) and transcription termination sites (TTSs) are preferentially located at the boundaries of the TAD-like domains, while pseudogenes and multigenic family genes are located in unstructured regions of the genome. Our data indicate 3D clustering of tRNA loci, likely optimizing transcription by RNA polymerase III, and a complex interaction between spliced-leader RNA and 18S rRNA loci. Our findings provide insights into 3D genome organization in *T. cruzi*, contributing to the understanding of supranucleosome-level chromatin organization and suggesting possible links between 3D architecture and gene expression. We propose an analogy to the art of origami, where papers can be folded into various shapes, resembling the DNA packed in chromatin fibers that also assume distinct folds within the nucleus.

35 (TT) High-accuracy genome assembly reveals hotspots for large gene families in *Trypanosoma cruzi* chromosomes

Lissa Cruz-Saavedra (McGill University); Luiza Berenguer Antunes (McGill University); Mira Loock (McGill University); Igor Cestari (McGill University)

Trypanosoma cruzi exhibits high genomic plasticity characterized by aneuploidies and repetitive sequences encoding virulence factors. The complexity of this parasite genome and the diversity of strains pose challenges for reconstructing the complete nuclear genome, resulting in limited high-quality reference genomes. Multigene families, such as mucins and trans-sialidases, play a critical role during infection; however, their genomic organization and expression throughout the parasite life stage are poorly understood. We generated a de novo assembly of *T. cruzi* Sylvio-X10 strain genome using long-read HiFi PacBio sequencing and chromatin conformation capture with nanopore sequencing (Pore-C). We assembled 35 complete chromosomes, 33 exhibiting telomeres, and resolved both haplotypes. We found 17 megabase (Mb) size chromosomes, 14 varying between 0.5-1 Mb, and 4 smaller than 0.5 Mb. Moreover, 34 chromosomes are diploid, and one Mb-size chromosome is tetraploid. There is evidence of segmental aneuploidy in various chromosomes, indicating the plasticity of this parasite genome. We identified 13,579 genes, of which 36% are large multigene families annotated at the subtype level. Notably, two Mb-size chromosomes have ~80% of their sequences represented by repeats, mainly large multigene families, suggesting a hotspot for their diversification. Retrotransposons and the Disperse Gene Family were enriched at subtelomeric regions, whereas other multigene families were compartmentalized across chromosomes. Gene expression analysis by RNAseq and tandem mass tag labelling mass spectrometry are in progress for each *T. cruzi* life stage. The data provide hints on *T. cruzi* genome organization and the complexity in the distribution and expansion of large gene families.

36 (TT) Cross-linking and mass spectrometry uncovers chromatin compartment proteins and subtelomeric VSG gene silencing

Tony Isebe (McGill University); Luiza Antunes (McGill University); Oksana Kutova (McGill University); Igor Cestari (McGill University)

Chromatin organization is essential in regulating gene expression in eukaryotes. In *Trypanosoma brucei*, chromosomes are organized into RNA polymerase II transcribed regions (core) and subtelomeric non-transcribed regions. This parasite has over 2,500 VSG genes and pseudogenes spread throughout subtelomeric regions used for VSG recombination during antigenic variation. We found that the enzyme phosphatidylinositol 5-phosphatase (PIP5Pase) and repressor-activated protein 1 (RAP1) interact and function in silencing VSGs from telomeric expression sites. However, the mechanisms by which hundreds of subtelomeric VSG genes are silenced remain unknown. Using in vivo chemical cross-linking, immunoprecipitation, and mass spectrometry, we mapped the interactions of PIP5Pase, RAP1 and their co-interacting proteins at peptide resolution within a radius of 11 Å. The data comprises over 10,000 crosslinked peptides. It revealed a large network of protein associations, including kinetochore, histones, cohesin complex, and chromatin-modifying enzymes, identifying their binding domains and revealing proteins that mediate multivalent interactions. Chromatin conformational capture (Hi-C) and ChIP-seq showed that RAP1 is enriched at the boundaries of chromosome compartments, often overlapping with sister chromatid cohesin (SCC1) and H3.V, indicating that interactions reflect protein composition at defined chromosome locations. Moreover, RAP1 spread over silent subtelomeric regions but not to transcribed chromosome core regions. PIP5Pase knockdown or mutation that inhibits PI(3,4,5)P3 dephosphorylation resulted in RAP1 removal from subtelomeric VSG genes and led to transcription of subtelomeric genes. The data indicates a network of protein interactions at the chromosome compartment boundaries, likely coordinating chromosome three-dimentional organization, and provides a mechanism for silencing subtelomeric VSG genes.

DISCUSSION - Abstracts

Present and future of pathogen genome resources

Chair: Luisa M Figueiredo, Chris de Graffenried and Megan Povelones

Thursday, September 12

1:45PM – 2:15PM

37 TriTrypDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration, Discovery, and Dissemination

David S. Roos (Univ Pennsylvania… on behalf of the VEuPathDB Bioinformatics Resource Center)

Biomedical research is increasingly driven by Big Data: genome sequences and population-level diversity data, all manner of multi-Omics datasets, genome-scale phenotypic analyses, *etc*. How can we effectively collect, store, maintain, integrate and share this information to ensure FAIR (**F**indable, **A**ccessible, **I**nteroperable, **R**eusable) data access, advancing biological understanding and defining targets for further study in the lab, field and clinic? Over the past 25 years, the Eukaryotic Pathogen and Vector Bioinformatics Resource Center (VEuPathDB.org) – including TriTrypDB.org – has provided a robust, scalable, data-mining resource, accessed by thousands of researchers daily to inform and expedite discovery research and translational applications involving diverse eukaryotic microbes (fungi & protists) and their interactions with host/vector species. These resources offer:

- Information on genes, gene models, automated & curated annotation, genomes, population diversity, comparative genomics, epigenetics, transcriptomes, proteomes, DNA & protein motifs, protein structures, interactomes, subcellular localization, metabolomics, pathways, genotype association with fitness and other phenotypes, orthology-based functional inference, *etc*
- Strategies for integrating & interrogating diverse datasets (*in silico* experiments) … and analyzing & sharing the results obtained
- Assessment and improvement of available annotation … capturing expert knowledge from the community
- Analysis of user-provided (or any publicly available) datasets … which may be explored in the context of other data in VEuPathDB, and shared in compliance with emerging data-sharing standards/requirements

Additional functionalities recently released or under development include support for new datatypes (*e.g.* long-read & scRNAseq data), dataset comparisons (*e.g.* correlation of host-pathogen interactions), algorithms (*e.g.* for improved orthology detection), and AI-driven improvements to metadata annotation, literature curation, and database queries.

38 The NIAID Bioinformatics Resources Centers (BRCs) for Infectious Diseases: accelerating bioinformatics for parasites, bacterial, fungal, vector, and viral infectious disease research

Liliana Brown (NIAID); Wiriya Rutvisuttinunt (NIAID)

NIAID supports bioinformatics research through the NIAID Bioinformatics Resource Centers (BRCs) to offer online resources for pathogens, vectors, and their hosts. The FY24 BRCs program will have four main activities: (1) provide integrated data and bioinformatics resources; (2) develop advanced, innovative bioinformatics, software, and tools for basic and applied infectious diseases research; (3) offer bioinformatics trainings, educational materials, and other community outreach activities in the US and globally; (4) offer bioinformatics resources and analytics in response to emerging needs, outbreaks, and public health emergencies. The FY24 BRCs will implement advances in bioinformatics infrastructure for uniform and harmonized access to pathogen-relevant external resources, integration of infectious diseases with additional human and clinical data, and large-scale automated workflows and dataset management. Analytical and functional innovations in the BRCs include application of artificial intelligence (e.g. LLM and ML) to enrich bioinformatics services, improve genotype to phenotype and functional predictions, more accurate pathogen genome annotation, deployment of large-scale protein structural modeling, and small-molecule screening for vaccine and drug development for all pathogen types. The BRCs will embrace a decentralized paradigm incorporating globally distributed repositories and analytical capabilities that will be strengthened by program-wide commitment to FAIR principles and collaborative work. Together, the BRCs will develop criteria for data ownership, sharing, governance, and trust across the thousands of pathogen data generators globally. Last, the BRCs will enhance NIAID's outbreak and pandemic preparedness by offering platforms that integrate public health, pathogen and other and heterogenous host data to high-quality open-source tools, prior to and during outbreaks.

Session V – Abstracts

The host perspective

Chair: Federico Rojas

Friday, September 13

8:45AM – 11:30AM

39 (Plenary) Time to consider *Trypanosoma cruzi*

Rick L. Tarleton (Center for Tropical and Emerging Global Diseases, University of Georgia)

Of the "tri-tryps", *Trypanosoma cruzi* is the least-studied and the poorest understood of the trio. The reasons for this are multiple and complicated, but are frequently based on misinformation. Certainly, the lack of interesting questions in not one of the reasons that *T. cruzi* is avoided. One of the charges I have been given for this talk is to encourage investigators to choose *T. cruzi* as a research focus. Toward that end, I will try to clarify briefly what we know about *T. cruzi* and Chagas disease, focusing on its unique aspects relative to the other tri-tryps and identifying some of the areas of need. There are very good practical reasons for working with *T. cruzi*; it remains one of the highest impact infectious diseases in the americas and is the major infectious cause of cardiac disease worldwide. In essentially all of the many mammalian species that it infects, *T. cruzi* is controlled immunologically but often not eliminated but instead persists by replicating primarily in muscle tissues. We believe we are close to understanding why *T. cruzi* is so challenging for the immune system to eliminate. Among *T. cruzi*'s multiple immune evasion mechanisms is its collection of large families of surface protein that are highly variant among strains and which are undergoing constant diversification. Among the tri-tryps, *T. cruzi* probably has the most freedom with respect to genome rearrangements and we suspect that the transiently dormant amastigotes which create a challenge for drug treatment are a result of a stalled DNA repair process. The endemicity of *T. cruzi* in the U.S. and the accessibility of naturally acquired infections that occur in non-human primates and dogs, has recently allowed for the rapid progression of highly effective new drugs and for a clearer understanding of the diversity of infection control that occurs in individual animals, among other insights and developments. Indeed, the high quality and accessibility of non-human species with naturally acquired *T. cruzi* infection presents research opportunities that are difficult to match in any other parasite species.

40 Identifying vaccine targets for Chagas disease using yeast surface display screens

Mira Loock (McGill University); Valeria B Araujo (McGill University); Luiza B Antunes (McGill University); Igor Cestari (McGill University)

Chagas disease (ChD) affects roughly 8 million people and is caused by infection with the protozoan parasite *Trypanosoma cruzi*, resulting in 12,000 annual deaths. The disease begins with an acute infection presenting mild flu-like symptoms and then progresses to the chronic, typically lethal, cardiac stage. There are no vaccines available against ChD and existing drugs – benznidazole and nifurtimox – are highly toxic and not effective at the chronic stage. To address the lack of vaccine targets, we developed a *T. cruzi* genome-wide library for yeast surface display (YSD) to screen for immunogenic antigens in humans. Nanopore sequencing of the library showed complete coverage of the parasite genome, expressing over 240,000 unique polypeptides. We confirmed successful yeast surface protein expression by flow cytometry, microscopy, and Western blot. To identify antigenic polypeptides, we screened the YSD library against antibodies from chronic ChD patients or healthy donors via magnetic-activated cell sorting. Nanopore sequencing of enriched libraries identified ~1700 antigens ($p<0.05$; fold-change > 2) and defined immunogenic regions at nucleotide resolution. 829 antigens were exclusively enriched with antibodies from ChD patient serum vs healthy individuals. About 430 antigens were conserved among *T. cruzi* DTUs and do not belong to variable gene families and thus are potential vaccine candidates. Selected antigens have been validated by re-expression in yeast and flow cytometry with the Chagas patient's antibodies, confirming their antigenicity. Vaccination studies in mice with recombinant proteins and mRNA will be performed to determine if antigens confer protection against *T. cruzi* infection.

41 Fexinidazole induced cytotoxicity is distinct from related anti-trypanosome nitroaromatic drugs

Kenna Berg (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Indea Rogers (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Hayley Ramirez (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Ignacio Durante (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Julian Cornejo (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Paprika Berry (George Washington University School of Medicine and Health Sciences Department of Microbiology, Immunology, and Tropical Medicine); Galadriel Hovel-Miner (Stony Brook University, Renaissance School of Medicine, Department of Microbiology and Immunology)

Fexinidazole is the newest member of clinically significant anti-Trypanosomatid drugs classified as nitroaromatics. Nifurtimox and benznidazole have been used to treat American trypanosomiasis for decades and more recently the use of a combination therapy including nifurtimox and eflornithine (NECT) is a critical therapy for Human African Trypanosomiasis (HAT). Now, fexinidazole has been added as the first oral monotherapy against HAT. This presents enormous new therapeutic potential. However, much remains to be understood about fexinidazole. Nitroaromatic drugs are activated in trypanosomes by a type I nitroreductase (NTR), whose mutations pose a potential source of naturally occurring drug resistance. NTR activation of nifurtimox results in the formation of reactive open chain nitriles and benznidazole is activated to form guanidine and toxic glyoxal forms. The outcomes of fexinidazole activation have not been fully elucidated but the drug is expected to be NTR activated. While it is unclear precisely how any of the nitro-drugs kill parasites, proposed mechanisms include ROS activation and the induction of DNA damage. Here, we evaluate the cytotoxic outcomes of nitroaromatic drugs on Trypanosome brucei in vitro and focus on how nitroaromatic drug treatments impact the related the outcomes of cell cycle, DNA damage, and DNA synthesis. Together these data comprise the most detailed analysis of nitroaromatic induced cytotoxicity to date. Of key importance we found that fexinidazole has distinct cytotoxic outcomes when compared with the related drugs. These observations will be the foundation of further studies to determine the drugs mechanism of action. In addition, we have identified a novel gene that promotes fexinidazole drug resistance and has the capacity to alleviate drug induced cytotoxicity. The mechanistic implications of this new drug resistance gene will also be discussed.

42 Resistance-associated mutations in the target of acoziborole - trypanosome cleavage and polyadenylation specificity factor 3

Melanie C Ridgway (University of Dundee); Michele Tinti (University of Dundee); David Horn (University of Dundee)

Acoziborole is a safe, single dose, oral therapy, suitable for treatment of both early and late-stage human African trypanosomiasis. This drug is currently under development for paediatric application, while other oxaboroles show efficacy against Trypanosomatids, Apicomplexans and fungi. Acoziborole and several other oxaboroles target cleavage and polyadenylation specificity factor 3 (CPSF3; see PMID: 30185555). We developed oligo targeting for rapid and precision editing in otherwise wild type Trypanosomatids (PMID:35524555) and subsequently found that the method could be used to introduce multiple edits in one step. We've now used the approach for saturation mutagenesis around the drug-binding, and CPSF3 catalytic pocket, and for multi-editing. Among >1000 edits affecting residues within 5 Å of the acoziborole binding site, only the pair of Asn232His edits conferred moderate resistance to acoziborole or the related oxaboroles, DNDi-6148 and AN3661. By targeting multiple codons simultaneously, however, we found additive or synergistic acoziborole resistance-conferring edits. Together with a Asn232His edit, Tyr483Asn, and Asn448 edits increased resistance relative to the Asn232His edit alone. These multi-edited sites are homologous to AN3661 resistance-conferring mutations described in *Plasmodium falciparum* and Toxoplasma gondii. This study highlights the versatility of oligo targeting and provides new insights into CPSF3-associated acoziborole resistance.

43 The local immune response to *Trypanosoma brucei* in the tissues of the abdomen

Chloe Barnes (Lancaster University); Megan Dooley (Lancaster University); Nadia Iqbal (Lancaster University); Alex Hardgrave (Lancaster University); Sheila Macharia (Lancaster University); Ivo Basilio-Queijo (Lancaster University); Otto Wheeler (Lancaster University); Neil Dawson (Lancaster University); Lucy Jackson-Jones (Lancaster University); John Worthington (Lancaster University); Mick Urbaniak (Lancaster University)

Trypanosoma brucei, can survive and develop a chronic infection in the mammalian host despite eliciting a strong anti-trypanosome immune response. The most severe infection symptoms are associated with the late-stage infection and parasite colonisation of the brain. However, parasite tissue niches in early infection are increasingly appreciated to have clinical importance particularly those in the skin and adipose tissue. A bioluminescent in vivo model of African trypanosomiasis was used to observe tissue tropism during infection. Trypanosomes were identified at a high burden within the large intestine and omentum. Analysis of the large intestine showed barrier inflammation with increased crypt pathology in histological staining of infected sections. We observed significant enlargement of the omentum, a specialised immunological adipose tissue located in the peritoneal cavity which captures contaminants that can enter the peritoneal space from the gut. Changes in immune cell populations of the large intestine lamina propria and mesenteric lymph nodes were observed, along with alterations in host gene expression levels in the gut and omentum shown by bulk RNA sequencing. Our results suggest parasite localisation to the gut may explain reports of endotoxemia observed in humans and cattle infected with African trypanosomes. Adipose tissue loss is widely reported in experimental African trypanosomiasis, with weight loss and cachexia also observed in patients and cattle respectively. Mice were fed a high fat diet prior to trypanosome infection, resulting in reduced parasite burden across numerous tissues, suggesting that lipids are utilised to fuel the anti-trypanosome immune response to the benefit of the host.

44 Understanding weight loss in mice infected by *Trypanosoma brucei*

David Ferreira (Instituto de Medicina Molecular); Henrique Machado (Instituto de Medicina Molecular); Sandra Trindade (Instituto de Medicina Molecular); Abdulbasit Amin (Instituto de Medicina Molecular); Luisa M Figueiredo (Instituto de Medicina Molecular)

Trypanosoma brucei infection causes weight loss in humans and cattle. In mice, this parasites leads to loss of 70% of fat mass, coinciding with a significant migration and accumulation of parasites in adipose tissue. Our lab recently demonstrated that this fat mass loss is primarily driven by increased adipocyte lipolysis, mediated by the ATGL enzyme. To investigate the activation of adipocyte lipolysis, we used MRI to monitor body fat mass during an infection of mice that lack B and T cells (RAG2-/-). These mice retained significantly more fat mass than wild-type mice, indicating that the adaptive immune response plays a role in lipolysis activation. Unexpectedly, mice deficient for TNF-α, a known activator of adipocyte lipolysis, showed only a slight delay in fat loss, suggesting additional inflammatory factors are involved. Notably, co-culturing adipocytes with *T. brucei* also triggered lipolysis without requiring cell-to-cell contact, suggesting that parasites secrete molecules that stimulate host cell lipolysis. We propose that fat mass loss is driven by systemic signals from the inflammatory response and local signals originating from the parasite in the adipose tissue.

45 (TT) Genome analysis of *T. cruzi* field isolates offers the opportunity to study the effect of infection context on parasite genetic diversity

Jill Hakim (Johns Hopkins); Sneider Gutiérrez (Johns Hopkins); Edith Malaga (Universidad Cayetano Herida); Louisa Messenger (University of Arizona); Monica Mugnier (Johns Hopkins)

Trypanosoma cruzi is the causative agent of Chagas disease which kills 10,000 people annually. There has been little investigation into the parasite's highly adaptive genome, especially in strains infecting humans. Characterizing the genomes of clinical strains is critical to defining the role that the human host plays in the parasite's evolutionary adaptation. Here, we describe short read whole genome sequencing of 15 clinically isolated *T. cruzi* samples from different infection contexts: mothers at time of delivery, patients with Chagasic cardiomyopathy, and patients co-infected with HIV. We have produced gene level assemblies for each sample and categorize parasites into canonical discrete typing units (DTUs). Additionally, we uncovered clusters of genetically similar parasites within DTU groups, indicating underlying genetic population structures not captured by DTUs that may be explained by other, perhaps epidemiological or geographic, variables. Of particular interest are diverse and highly antigenic multi-copy gene families, which are understudied yet represent important diagnostic targets. We find these families are expanded and more diverse in clinically isolated specimens compared to lab adapted strains. Moreover, we find a repertoire of genes for each multi-copy gene family member found across all clinical isolates, but are found in none of the lab adapted strains, regardless of DTU. Finally, to identify genes that may be under purifying selection during human infection, we calculated pN/pS within each sample and across samples. Genes with high pN/pS across all samples include members of ABC transporter complex, intraflagellar transport protein 88, and other important genes. This represents the first comparison of whole genomes from such a wide array of clinical contexts. These results demonstrate the feasibility of large-scale *T. cruzi* whole genome studies, allowing further investigation into genetic features driving clinical manifestation of disease.

46 (TT) Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models

Sara Fresard (CUNY Hunter College, The Graduate Center at City University of New York); Kayla Leiss (CUNY Hunter College); Russell Thomson (CUNY Hunter College); Jayne Raper (CUNY Hunter College, The Graduate Center at City University of New York)

African Trypanosomes cause a major agricultural and economic burden due to their negative impact on cattle. Some primates are protected against 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)), for parasite receptor-mediated endocytosis. How these TLFs are assembled is unknown. To elucidate TLF assembly, we analyzed human serum, hepatocyte tissue cell culture, and generated targeted germline transgenic murine models. We used immunoaffinity and size exclusion chromatography to isolate all APOL1 complexes from serum. TLF 1 and TLF2 were isolated as well as a small TLF ~150 kDa complex, TLF3. We hypothesize that TLF3 is a nascent HDL synthesized in hepatocytes based on its size, density, lytic ability, and gene expression. In our transgenic TLF mice, the most robust protection from trypanosome infection is observed when APOL1 and HPR are expressed from ubiquitin promoters, supporting the idea that TLF is made and secreted by cells under the same conditions, perhaps the same cell. To test this, we evaluated the secreted proteins from a human hepatocyte cell line (HepG2). We detected robust co-assembly of APOL1 and APOA-I (HDL scaffold protein) by size fraction and immunoaffinity purification. We detect low levels of Hpr and hypothesize that APOL1, and sometimes HPR, are loaded onto HDL in/on the hepatocyte with sufficient lipids to generate a nascent HDL. The TLF3 complex is released into the blood and matures into TLF1 by accumulating lipids and more HPR. By understanding TLF biogenesis, we can use the appropriate promoters in transgenic models to generate cattle completely resistant to trypanosomiasis.

47 (TT) Host response to cutaneous Human African Trypanosomiasis

Rhiannon Heslop (University of Glasgow); Nono-Raymond Kuispond Swar (National Institute for Biomedical Research, Kinshasa); Anneli Cooper (University of Glasgow); Paul Capewell (University of Glasgow); Dieudonne Mumba Ngoyi (National Institute for Biomedical Research, Kinshasa); Annette MacLeod (University of Glasgow)

Dermal trypanosomes have been identified in both Gambiense Human African trypanosomiasis (gHAT) patients, who have bloodstream parasites, and asymptomatic seropositive individuals, who lack detectable bloodstream parasites. However the prevalence of cutaneous trypanosomiasis and how these parasites persist in the skin remains unknown.

Here we have serologically screened 123 individuals from gHAT foci in the DRC. Blood samples and skin biopsies were then examined for evidence of trypanosomes by molecular and immunohistological methods. RNAseq analysis was conducted on 30 skin biopsies from seropositive and seronegative individuals to investigate the host dermal response to trypanosome infection.

We found 64% of seropositive individuals had trypanosomes in their skin. Our cohort included 19 individuals with a previous gHAT diagnosis, of whom 14 had trypanosomes in their skin up to 13 years after clinically successful treatment. Host transcriptomic analysis of skin biopsies revealed that individuals clustered by presence of dermal trypanosomes. Expression patterns compared between uninfected (serology and histology negative) and infected (serology and histology positive) individuals detected 1113 differentially expressed genes and 170 enriched pathways which were predominantly involved in infection, inflammation, cell proliferation and barrier integrity.

Our results demonstrate that cutaneous trypanosomiasis is widely prevalent in gHAT foci and that ‘successful’ treatment does not guarantee the clearance of dermal trypanosomes. This highlights that skin analysis could be a valuable addition to clinical diagnosis and treatment monitoring of gHAT. This pilot study shows a clear local host response to dermal trypanosomes and identifies candidates for biomarkers that could be used in future gHAT diagnosis.
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Session VI – Abstracts

Insights from structures

Chair: Stella Sun

Friday, September 13

2:00PM – 4:45PM

48 Structural and functional insights into ESAG3, a putative glycosyltransferase, in African trypanosomes

Qi Zhong (Imperial College London); Joseph D Barritt (Imperial College London); Carla Gilabert Carbajo (Imperial College London); Emmanuel Nji (Centre for Research in Infectious Diseases); Samuel Dean (University of Warwick); Michele Tinti (University of Dundee); Michael A.J. Ferguson (University of Dundee); Erhard Hohenester (Imperial College London); Sarah Rouse (Imperial College London); Calvin Tiengwe (Imperial College London)

African trypanosomes are highly effective extracellular parasites that evade host immune responses by antigenic variation of variant surface glycoproteins (VSGs). VSGs are co-transcribed alongside expression site-associated genes (ESAGs) that play key roles in host adaptation, including nutrient acquisition and resistance to human serum. However, the specific functions of most ESAG proteins remain unknown. In our search for iron-responsive factors in *Trypanosoma brucei*, we found that ESAG3 was co-upregulated with the transferrin receptor (ESAG6/7) during iron starvation. ESAG3 has a predicted molecular weight of 42.5 kDa, an N-terminal signal sequence, and six potential N-glycosylation sites. Structural predictions indicate that ESAG3 shares conserved features of class-A glycosyltransferases (GT). Here, we show that RNAi-mediated depletion of ESAG3 inhibits parasite growth in vitro. ESAG3 localises to the ER, contains both endoglycosidase H-sensitive and endoglycosidase H-resistant N-glycans, and forms higher-order oligomers of ~700 kDa in trypanosome cell extracts. We resolved the structure of ESAG3 by single-particle cryo-electron microscopy at 3.2 Å. ESAG3 forms a ring-shaped homo-octadecameric structure arranged into three hexamers with C3 symmetry. The fundamental building block is a dimer with two aromatic ring-rich GT active sites that anchor putative substrates. Additional density at position N99 protruding from the ring surface fits a mapped N-glycan, suggesting N-glycosylation occurs at this site. These findings provide the first high-resolution structure and domain organisation of a GT from Kinetoplastids. It is tempting to speculate that ESAG3 is the GT responsible for O-glycosylating trimeric VSGs, which also assemble into an octadecamer, for which the GT remains elusive.

49 Structural studies of CFAP410/C21orf2 reveal a bimodular organization with two distinct domains for its localization and function

Alexander Stadler (Medical University of Vienna); Laryssa V. De Liz (Oxford Brookes University); Heloisa Gabriel (Oxford Brookes University); Santiago Alonso-Gil (University of Vienna); Robbie Crickley (Oxford Brookes University); Katharina Korbula (Medical University of Vienna); Bojan Zagrovic (University of Vienna); Sue Vaughan (Oxford Brookes University); Jack D. Sunter (Oxford Brookes University); Gang Dong (Medical University of Vienna)

Cilia and flagella are antenna-like organelles protruding from the surface of most cells in the human body. Defects in ciliary structure or function often lead to diseases that are collectively called ciliopathies. Cilia and flagella associated protein 410 (CFAP410), which was originally named C21orf2, localizes at the basal body of cilia/flagella and plays essential roles in ciliogenesis, neuronal development and DNA damage repair. Multiple single amino acid mutations in CFAP410 have been identified in patients with two types of ciliopathies, spondylometaphyseal dysplasia, axial (SMDAX) and retinal dystrophy with or without macular staphyloma (RDMS). However, the molecular mechanism for how the mutations cause these disorders remains poorly understood due to a lack of high resolution structures of the protein. We have carried out extensive structural and biochemical investigations on *Trypanosoma brucei* CFAP410, as well as its homologs in Homo sapiens and Chlamydomonas reinhardtii, which result in four crystal structures in total. These structures together with our bioinformatics analysis reveal that CFAP410 is a bimodular protein comprising two distinct domains: a leucine rich repeat (LRR) motif at the N-terminus and a tetrameric helical bundle at the C-terminus. These two domains are interconnected via a long unstructured loop. We further examined how the disease-causing mutations may affect the folding and/or assembly of CFAP410 using biochemical approaches and molecular dynamics simulation. Taken together with our in vivo localization data from *T. brucei*, we provide an explanation how the mutations in CFAP410 may cause retinal and skeletal ciliopathies.

50 Structures of mitochondrial RNA editing catalytic complexes from *T. brucei*

Ruslan Afasizhev (Boston University); Yun-Tao Liu (UCLA); Andres Vacas (Boston University); Jane Lee (UCLA); Xiaojing Zhao (Shanghai Tech); Jonathan Jih (UCLA); Takuma Suematsu (Boston University); Clinton Yu (UC Irvine); Lan Huang (UC Irvine); Liye Zhang (Shanghai Tech); Inna Afasizheva (Boston University); Hong Zhou (UCLA)

The discovery of RNA editing in trypanosomes challenged the central dogma of molecular biology and introduced fundamental biological entities, such as guide RNA (gRNA), editosome, and terminal uridyltransferases (TUTases). Although trans-acting RNAs underpin most modern genome and transcriptome-altering technologies, the mechanism of gRNA-programmed mitochondrial mRNA recoding has remained elusive. Here, we report molecular analyses and cryoEM structures of RECC1 and RECC2, the multienzyme complexes responsible for uridine deletion and insertion editing. Our results show that RECCs' distinct central bodies comprise active and inactive RNase III proteins that cleave mRNA in a duplex with gRNA. Heterotetramers of largely disordered oligonucleotide binding (OB) proteins flexibly dock exonuclease, TUTase, and ligase to the body, thereby facilitating trimming or extension of the cleavage product and mRNA backbone repair. An architectural tRNA locks the interface between RNase III and OB-fold subunits in both complexes. Although RECCs bind gRNA-mRNA hybrids in similar orientations, the asymmetry in their endonucleases' active centers directs mRNA splitting, while zinc finger domains distinguish deletion and insertion sites. Our findings illuminate the temporal and spatial order of the editosome assembly from substrate-binding RESC and catalytic RECC complexes, the structural basis of U-deletion and U-insertion enzymatic cascades, and the mechanism of mRNA scission.

51 Mechanistic Insights into KREH2C-Mediated gRNA Displacement in Trypanosomal RNA Editing

Shiheng Liu (University of California, Los Angeles); Takuma Suematsu (Boston University Medical Campus); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine); Liye Zhang (ShanghaiTech University); Inna Afasizheva (Boston University Medical Campus); Z. Hong Zhou (University of California, Los Angeles); Ruslan Afasizhev (Boston University Medical Campus)

In trypanosome mitochondria, uridine insertion/deletion RNA editing converts cryptic transcripts into functional messenger RNAs (mRNAs). RNA editing catalytic complex (RECC) executes a series of enzymatic reactions on gRNA/mRNA hybrid scaffolded by the RNA editing substrates binding complex (RESC). The editing process converts an imperfect gRNA hybrid with pre-edited mRNA into a ~50 bp nearly perfect duplex with edited mRNA. It follows that gRNA must be displaced to enable the binding of subsequent gRNAs during pan-editing and to allow translation of the fully edited transcript. The RNA Editing Helicase 2 Complex (KREH2C), consisting of the DEAH/RHA RNA helicase KREH2 and associated factors KH2F1 and KH2F2, has been implicated in the editing progression. However, the molecular mechanism of KREH2C action remains elusive due to the absence of high-resolution structures. Here, through cryo-electron microscopy and functional studies, we have discovered new factors and captured multiple states of KREH2C ranging from 0.4 to 0.7 MDa and determined their high-resolution structures. We will present KREH2C assembly dynamics and conformational changes underlying the gRNA translocation, a driving force of RNA editing complex remodeling.

52 Domain structure and function in RNA Editing Catalytic Complexes in *Trypanosoma brucei*

Jason Carnes (Seattle Children's Research Institute); Suzanne M. McDermott (Seattle Children's Research Institute); Brittney Davidge (Seattle Children's Research Institute); Tyler Rodshagen (Seattle Children's Research Institute); Isaac Lewis (Seattle Children's Research Institute); Maxwell Tracy (Seattle Children's Research Institute); Kenneth Stuart (Seattle Children's Research Institute)

RNA editing generates mature mitochondrial mRNAs in *T. brucei* by uridine insertion and deletion at editing sites specified by guide RNAs. Editing is performed by three RNA Editing Catalytic Complexes (RECCs) which have distinct endonucleases and 12 common proteins, resulting in deletion specific RECC1 and insertion specific RECC2 and RECC3. Thus, different RECCs are required to edit mRNAs that have both insertion and deletion sites. To elucidate the functions of RECC proteins, we generated and analyzed cells with single amino acid substitutions. Substitutions that eliminated or altered editing mapped to OB-fold, zinc finger, RNase III, RNase III Associated Motif domains, intrinsically disordered regions, and other regions in multiple non-catalytic RECC proteins. The various substitutions have different effects on the integrity of the complexes and on RNA editing, thus providing insights into the functions of the RECC protein domains. Several mutants had different consequences in bloodstream vs. procyclic life cycle stages. Studies that were performed in cells that express a mutant gamma ATP synthase allele (MGA) circumvented the essentiality of editing and thus avoided confounding secondary effects associated with either incomplete elimination of normal proteins or dying cells. Focused deep sequencing of mRNAs revealed greater impacts of the substitutions on editing in MGA cells than in non-MGA cells. Alteration of editing was observed in multiple mRNAs; however, different substitutions resulted in distinct RNA phenotypes. Overall, the results indicate that RNA editing requires the collaboration of multiple distinct RECC protein domains to ensure RECC integrity, editing site recognition and processing.

53 The small nucleolar RNAs are master regulators controlling ribosome structure and function and mRNA fate in trypanosomes and *Leishmania*

K Shanmugha Rajan (Weizmann Institute); Saurav Aryal (Bar-Ilan University Beathrice); Beathrice Galili Kostin (Bar-Ilan University); Anat Bashan (Weizmann Institute); Mika Olami (Weizmann Institute); Hava Madmoni (Bar-Ilan University); Sharanya Murugeshan (Bar-Ilan University); Tirza Doniger (Bar-Ilan University); Smadar Cohen-Chalamish (Bar-Ilan University); Ron Unger (Bar-Ilan University); Christian Tschudi (Yale university); Gerald F . Späth (Pasteur Institute); Ada Yonath (Weizmann Institute); Shulamit Michaeli (Bar-Ilan University)

snoRNAs are master regulators in Trypanosomatids controlling ribosome function and mRNA fate. SnoRNA belong to the C/D family guiding 2'-O-methylation (Nm) and H/ACA RNA guiding pseudouridylation on rRNA and small non-coding RNAs. Trypanosomes and *Leishmania* genomes encode for ~200 snoRNAs (~80 H/ACA and 120 C/D). Both pseudouridines and Nms were shown to be developmentally regulated in both *T. brucei* and *Leishmania*. Recent studies from our groups demonstrate that a change in a single pseudouidine H69 located in the large subunit present in the intersubunit bridge affected the translation of only subset of mRNAs due to specific changes in the structure of the ribosome (Rajan et al., Nat. Comm. 2023, Rajan et al., Cell Rep. 2024), suggesting that rRNA modifications contribute to the generation of specialized ribosomes. Here we will present data that *Leishmania* snoRNA functions as chaperone during ribosome biogenesis. Cryo-EM structure of ribosomes from cells overexpressing C/D snoRNA and its mutant version that cannot guide Nm at ~2.3-2.7 Å resolution suggested no structural changes in small subunit rRNA carrying the specific Nm modification. However, changes were observed in H68 of the large subunit rRNA due to a second base-pairing interaction, thus reviving the concept of chaperone activity for snoRNAs in ribosome biogenesis. We will present data that a subset of snoRNAs that guide modification on rRNA can also directly regulate the fate of thousands of mRNAs at the stability and translation level, suggesting that snoRNAs are master anti-sense regulators of RNA biogenesis even beyond the role of microRNAs in metazoan.

54 The Neddylation pathway is a crucial regulator of *Trypanosoma brucei* differentiation

Federico Rojas (Lancaster University)

Differentiation in *Trypanosoma brucei* is an essential process that enables these parasites to adapt to the changing environments inside the host and the vector. Protein changes during differentiation in *T. brucei* are dynamic and tightly regulated. Neddylation is a post-translational modification (PTM) process involving the covalent attachment of the ubiquitin-like protein NEDD8 to target proteins. This modification can play a regulatory role in various cellular processes, including protein stability and degradation, cell cycle regulation and signal transduction. NEDD8 activates the E3 ubiquitin ligase family, the Cullin-RING ligases (CRL). Cullin neddylation increases CRLs ubiquitination activity that optimise ubiquitination to target proteins. This process is driven by DCN1, an E3 ligase essential in humans. Conversely, Cullin de-neddylation is managed by the COP9 signalosome (CSN), a de-neddylase complex. In *T. brucei*, the knock-out of the TbDCNL1 homologue shows delayed differentiation in mice compared to wild-type cells. Knock-out of two CSN subunits of *T. brucei* leads to premature differentiation. Interestingly, these KOs do not exhibit in vitro growth impairment, underscoring the exclusive involvement of these proteins in cell transformation and emphasising the critical role of the NEDD8 pathway in parasite differentiation. To identify the *T. brucei* Neddylome, total Ty1-NEDD8-modified proteins were immunoprecipitated from slender and stumpy cells and analysed by LC-MS. Known differentiation regulators of differentiation were identified as substrates of NEDD8. This study uncovers significant new insights into *T. brucei* differentiation regulation driven by protein Neddylation, while also identifying unknown NEDD8 substrates of eukaryotic cells and illuminating the regulatory impact of this PTM.

55 (TT) RNA viruses in *Blastocrithidia*: does a unique genetic code protect from viral infection?

Danyil Grybchuk (University of Ostrava); Donnamae Klocek (University of Ostrava); Alexei Kostygov (University of Ostrava); Vyacheslav Yurchenko (University of Ostrava)

Trypanosomatids (Euglenozoa) are a diverse group of unicellular flagellates predominately infecting insects (monoxenous species) or circulating between insects and vertebrates or plants (dixenous species). Monoxenous Trypanosomatids harbor a wide range of RNA viruses belonging to the families Narnaviridae, Totiviridae, Qinviridae, Leishbuviridae, and a putative group of tombus-like viruses. In this work, we focus on the subfamily Blastocrithidiinae, a previously unexplored divergent group of monoxenous Trypanosomatids comprising two related genera: Obscuromonas and *Blastocrithidia*. Members of the genus *Blastocrithidia* employ a unique genetic code, in which all three stop codons are repurposed to encode amino acids, with TAA also used to terminate translation. Obscuromonas isolates studied here bear viruses of three families: Narnaviridae, Qinviridae, and Mitoviridae. The latter viral group is documented in Trypanosomatid flagellates for the first time. Importantly, no RNA viruses were detected in *Blastocrithidia* spp., arguing that recoded genetic code indeed provided a protection against viral infections. Nevertheless, we identified an endogenous viral element in the genome of B. triatomae indicating its past encounter(s) with tombus-like viruses.

56 (TT) REH2C Complex is the First Identified Regulatory Factor in Lifecycle Stage-specific RNA Editing Repression During *Trypanosoma brucei* Development

Cody Goode (Texas A&M University); Binny Kaur (Texas A&M University); Sunil Sharma (Texas A&M University); Zihao Chen (University of Edinburgh); Tyler Rodshagen (Seattle Children's Research Institute); Scott Grote (Harvard Medical School); Joshua Meehan (Texas A&M University); Al Ivens (University of Edinburgh); Nick J. Savill (University of Edinburgh); Silvi Rouskin (Harvard Medical School); Achim Schnaufer (University of Edinburgh); Suzanne M. McDermott (Seattle Children's Research Institute); Jorge Cruz-Reyes (Texas A&M University)

U-indel RNA editing in procyclic-form (PCF) and bloodstream-form (BSF) *Trypanosoma brucei* is developmentally regulated; however, the mechanisms and factors driving this regulation have remained a critical open question since RNA editing was discovered. Editing directed by anti-sense gRNAs creates canonical protein-encoding mRNAs. Intriguingly, canonical editing occurs amid massive alternative non-canonical editing of unclear biological significance. The complex REH2C includes helicase KREH2 and its binding partners KH2F1 and KH2F2. We report here that REH2C is crucial for stage-specific editing control. Specifically, KREH2 promotes PCF- and BSF-specific editing repression at major early checkpoints in mRNAs encoding ND7 and cytochromes COX3 and CYb. KREH2-mediated repression involved opposite modulation of canonical and alternative "terminator" gRNA utilization, which derails canonical editing. In vitro differentiation recreated stage-specific repression. ND7 studies showed terminator-programmed editing installs a proposed repressive structure determined using DMS-MaPseq. KREH2-RNAi knockdown reverted canonical/terminator gRNA utilization and thus the repression phenotype. Transcripts that bypassed early termination were still repressed further upstream by a similar mechanism, suggesting global modulation of gRNA utilization. Terminators are novel "moonlighting" gRNAs also associated with canonical editing in their cognate mRNAs, reveling that the gRNA transcriptome is multifunctional. Notably, changes in the level of KH2F1 and KH2F2, which directly bind KREH2, can dramatically affect stage-specific editing repression. KREH2 is the first identified repressor in developmental editing control. This and our prior work support a model whereby KREH2 activates or represses editing in a stage and substrate-specific manner. KREH2's novel dual role tunes mitochondrial gene expression in either direction during development.

57 (TT) Deep mutational resistance profiling for anti-Trypanosomal proteasome inhibitors

Simone Altmann (University of Dundee); Michele Tinti (University of Dundee); Melanie Ridgway (University of Dundee); Manu de Rycker (University of Dundee); Michael Thomas (University of Dundee); Cesar Mendoza Martinez (University of Dundee); Jagmohan Saini (University of Dundee); Peter Ibrahim (University of Dundee); Mike Bodkin (University of Dundee); David Horn (University of Dundee)

Although anti-infective drug resistance presents a major threat, characterisation of potential resistance-associated mutations often remains incomplete. Now that several new anti-Trypanosomal drugs, with known targets, are in clinical development, we aim to improve our understanding in this area. We developed oligo targeting for precision editing in otherwise wild-type Trypanosomatids (PMID:35524555) and have now scaled the approach for saturation mutagenesis of residues comprising a drug-binding pocket. The *Trypanosoma brucei* proteasome is currently a promising anti-Trypanosomal target (PMID:27501246, PMID:30962368), and twenty residues within 5Å of bound drug in cryo-EM structures were targeted for saturation mutagenesis, stepwise drug-selection, and amplicon-sequencing. Among 1,280 mutants in the pooled library, codon variant scoring revealed resistance 'hotspots', which aligned well with 'functional' mutational space, as determined by fitness profiling; edits of residues directly involved in catalysis failed to yield survivors, for example. Nevertheless, >100 distinct resistance-conferring base-edits and >45 distinct amino acid edits were recovered. This contrasts with only a small number of single nucleotide polymorphisms recovery following drug-selection without editing, providing insights into limits imposed within 'accessible' mutational space. The digital data yielded virtual dose-response curves, which were predictive of EC50 values derived in vitro using a bespoke panel of edited mutants (R2 = 0.98); resistance increased up to 100-fold relative to the 4 nM EC50 observed for wild-type cells. Iterative computational modelling, informed by the quantitative experimental data, revealed how specific steric constraints, charge differences and backbone interactions contributed to varying degrees of resistance. The methods and findings we describe have the potential to facilitate modelling of drug-target interactions, assessment of drug-resistance potential, and design of more efficacious and durable drugs.

58 (TT) Forging the One Ring of *Trypanosoma brucei*: Structural characterization of BILBO1 assembly

Kim Ivan Abesamis (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter); Arda Kara (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter); Derrick Robinson (Microbiologie Fondamentale et Pathogénicité, CNRS UMR5234, University of Bordeaux); Mélanie Bonhivers (Microbiologie Fondamentale et Pathogénicité, CNRS UMR5234, University of Bordeaux); Gang Dong (Max Perutz Labs, Medical University of Vienna, Vienna Biocenter)

The Flagellar Pocket (FP) of *Trypanosoma brucei* is essential for the parasite's survival and pathogenicity. It functions as the sole site for all endo- and exocytosis activities and is crucial in the clearance of host antibodies. The FP's biogenesis and maintenance rely heavily on the flagellar pocket collar (FPC), a ring-like structure located at the neck of the FP. The assembly of the FPC requires BILBO1, a multi-domain cytoskeletal protein. Previous studies show that RNAi depletion of BILBO1 causes cellular abnormalities, including the lack of new FP and FPC, leading to cell death. However, the mechanisms by which BILBO1 forms the FPC scaffold and regulates FPC assembly remain unclear. To explore this, we conducted structural studies on full-length and truncated BILBO1. We determined a 1.9-Å resolution crystal structure of the BILBO1 filament junction revealing that two leucine zipper motifs are arranged in an antiparallel fashion to connect neighboring BILBO1 coiled-coil dimers. This tight junction allows BILBO1 dimers to form long filamentous polymers. Electron microscopy of full-length BILBO1 showed that numerous filaments could laterally associate to form an extended belt-like structure, with globular domains aligned in registers to create distinct condensed stripes. Furthermore, calcium binding significantly modulates the rigidity and compactness of these stripes. Intriguingly, treating samples with a BILBO1-specific nanobody resulted in detaching filaments with loosely packed circular structures. Overall, these findings provide a mechanistic explanation for BILBO1 filament formation and bundling, offering a potential strategy for disrupting BILBO1 function in FPC assembly by targeting critical interfaces within the junction/bundles.

Session VII – Abstracts

Gene regulation

Chair: Danae Schulz

Saturday, September 14

8:45AM – 11:00AM

59 Challenging trypanosome antigenic variation paradigms using natural systems

Stephen D Larcombe (University of Edinburgh); Jane C Munday (University of Glasgow); Guy R Oldrieve (University of Edinburgh); COBALT Consortium

Although natural African trypanosome infections are sustained long-term in livestock hosts, most experimental studies of VSG surface antigen diversity have focused on mouse infections in the first waves of parasitaemia. We have compared VSG expression diversity during long-term infections of mice and cows with the same parasites (*T. brucei* EATRO 1125). We found parasites from cattle expressed a more diverse range of VSGs after establishment than those from mice. Longitudinal data from cattle showed that this diversity was maintained as infections progressed even at barely detectable parasitaemia. Moreover, in the chronic phase we rarely found “dominant” VSGs in either mouse or cows, with most expressed VSG comprising less than 1% of overall VSG expression. Analysis of the relatedness of expressed VSG (VSG orthogroups) highlighted that many orthogroups were only present in cattle, with the major orthogroup being a smaller proportion of the overall total. Interestingly, expression of similar VSGs with modified C-terminal domains was a feature of both mouse and cow infections. To understand the basis for VSG expression diversity, we analysed mouse infections using RAD51 and BRCA2 null mutants, which are impaired in homologous recombination (HR). Both mutants were capable of sustaining chronic infections but the diversity of expressed VSG was lower than WT parasites. These analyses confirm that VSG diversity is a consistent feature of trypanosome infections, including in a chronic bovine setting of relevance to field infections, and we establish that VSG switching can proceed with sufficient efficiency in the absence of HR to maintain long-term infections.

60 Novel expression-site body specific components finetune expression at the active-VSG expression-site in African trypanosomes

Lianne Lansink (University of York); Adam Dowle (University of York); Leon Walther (University of York); Joana Faria (University of York)

Antigenic variation is a highly sophisticated virulence mechanism, not fully understood in any organism. In *Trypanosoma brucei*, this process is rather extreme, whereby a single Variant-Surface-Glycoprotein (VSG) is expressed from a large genetic repertoire (>2600 genes) at very high levels. The active-VSG is transcribed by Pol-I within a sub-nuclear transcription factory, the expression-site-body (ESB), spatially proximal to several splicing bodies to enhance mRNA processing. Strikingly, only two ESB components have been identified in over twenty years since its discovery: ESB-specific-protein-1 (ESB1) and VSG-exclusion-protein-2 (VEX2), required for positive and negative regulation of VSG expression, respectively. Using TurboID combined with LC-MS/MS analysis, we identified three new ESB-specific components, alongside a range of ESB-enriched factors and five novel components of the surrounding splicing bodies. Using super-resolution microscopy, ESB-specific-protein 2 and 3 (ESB2 and ESB3) and ESB-associated-protein-1 (ESAP1) were shown to form a single sub-nuclear focus that colocalises with the ESB in bloodstream forms. Further, ESB2-3 are not expressed in insect stages, which do not undergo antigenic variation. In the mammalian-stage, we unravelled a complex network of co-dependencies between Pol-I/VEX1-2/ESAP1/ESB1-3, giving us a glimpse into the hierarchy of recruitment of these different factors to the ESB. Interestingly, RNAseq analysis following the depletion of either of the novel factors shows a specific role in regulation of expression-site-associated-genes (ESAGs) expression. It has long been known that ESAGs upstream of VSGs, despite co-transcription in the same polycistron, yield far fewer transcripts (>140-fold less), however, no factor directly responsible for this post-transcriptional control had previously been identified.

61 Repair template availability determines the VSG switching mechanism after a double-strand break in the active VSG in *Trypanosoma brucei*

Raúl O Cosentino (LMU Munich); Kirsty R McWilliam (LMU Munich); Zhibek Keneshkanova (LMU Munich); Atai Dobrynin (LMU Munich); Jaclyn E Smith (Johns Hopkins Bloomberg School of Public Health); Ines Subota (LMU Munich); Monica R Mugnier (Johns Hopkins Bloomberg School of Public Health); Maria Colomé-Tatché (LMU Munich); T. Nicolai Siegel (LMU Munich)

The ability of *T. brucei* to evade the host immune response is based on its capacity to switch the expression of its major surface antigen, the variant surface glycoprotein (VSG), in a non-random manner. The mechanisms driving the observed hierarchy in VSG expression remain elusive. A major challenge in unraveling this process has been the difficulty to track transcriptome changes and potential genomic rearrangements in single cells during VSG switching events. In this study, we present a highly sensitive single-cell RNA sequencing (scRNA-seq) approach tailored for trypanosomes that allowed us to track these changes. Our data show that following a double-strand break in the active VSG, the type of repair mechanism and the resulting newly active VSG depend on the availability of a homologous repair template in the genome. When such a template was available, repair proceeded through segmental gene conversion, creating novel mosaic VSGs. Conversely, in the absence of a suitable template, exclusively telomere-adjacent VSGs were recombined by break-induced replication. Moreover, in the latter scenario, a high proportion of cells switched VSG expression by activating a different telomeric expression site, but this population of cells faded out over time. Collectively, our results reveal the critical role of available repair sequences in the VSG switching and selection mechanism. Moreover, our study demonstrates the power of highly sensitive scRNA-seq approaches to detect genomic rearrangements that drive transcriptional changes at the single cell level.

62 Identification of a novel protein that suppresses VSG switching in *Trypanosoma brucei*

Navina Panneer Selvan (PHRI, Rutgers University); Aditi Mukherjee (PHRI, Rutgers University); Eliezer Cruz (IIBIO, UNSAM, San Martín, Argentina); Vanina Campo (IIBIO, UNSAM, San Martín, Argentina); Esteban Erben (IIBIO, UNSAM, San Martín, Argentina); Hee-Sook Kim (PHRI, DMMG, Rutgers University)

Trypanosoma brucei is a protozoan parasite that causes African trypanosomiasis. Mammalian infection occurs through the bite of an infected tsetse fly. The survival of *T. brucei* within the mammalian hosts relies on antigenic variation of its coat protein, Variant Surface Glycoprotein (VSG). The surface of the trypanosome is densely packed by VSG coat, which provides protection for the parasite but also triggers a strong host immune response. VSG is expressed strictly in a monoallelic manner from among 2,500 VSG archives in the genome of *T. brucei*. By periodically switching from one VSG to another, trypanosomes evade from adaptive immunity and survive. VSG coat switching is executed through transcriptional or recombinational mechanisms. We have identified and characterized a novel protein whose depletion led to a significant increase, ~150-fold, in the rate of VSG switching. From analyses of single-cell-cloned VSG switchers, we found that VSG switching occurred predominantly through VSG gene conversion. Reflecting this phenotype, we named the protein, SVS1 (Suppressor of VSG switching 1). This hyper-switching phenotype is interesting but puzzling, given that SVS1 localizes primarily in the cytoplasm (TrypTag) and has been identified from RNA bound proteome studies (Lueong et al 2016, Melo do Nascimento et al 2021). In addition to the hyper-switching, we recently found that lipidation of SVS1 may be important for its function. Here, we would like to discuss how a cytosolic SVS1 protein can control DNA recombination processes in the nucleus and possible roles for SVS1 lipidation.

63 Rab2B promotes chaperonin-mediated actin folding and prevents developmental transcription reprogramming and quiescence in trypanosomes

Feng-Jun Li (National University of Singapore); Cynthia Y. He (National University of Singapore)

The Rab family of small GTPases regulate intracellular membrane trafficking by facilitating the biogenesis, transport, tethering and fusion of membrane-bound organelles and vesicles. Recently, Rab2B, a Golgi-resident Rab protein, has been shown to play a role in autophagosome-lysosome fusion beyond its typical ER-Golgi transport function in mammalian cells and Drosophila. In *T. brucei*, the autophagy-related function of Rab2B was found to be well conserved: depletion of Rab2B leads to lysosome fragmentation and blocks autophagosome-lysosome fusion. More intriguingly, we found that Rab2B negatively regulates the differentiation of *T. brucei* from a proliferative long-slender form to a quiescent short-stumpy form, a life cycle step crucial for transmission of the parasites by the tsetse fly vector. Co-immunoprecipitation analysis revealed that chaperonin/CCT, which is essential for actin folding, interacts with Rab2B in a manner depends on its GTPase activity. In *T. brucei*, actin folding also requires CCT, and depletion of CCT or actin, or blocking actin polymerisation mimics Rab2B knockdown, inducing slender-to-stumpy differentiation. The nuclear pool of actin required for transcription was observed in *T. brucei* using actin chromobody, and degradation of nuclear actin leads to cell differentiation. These results shed light on a potential mechanism of Rab2B in transmitting environmental cues to the nuclear transcriptome shift and cell differentiation.

64 The enigma of PP-IPs in the *Trypanosoma cruzi* life cycle

Thaise Lara Teixeira (University of São Paulo); Bryan Abuchery (University of São Paulo); Vitor Luiz da Silva (São Paulo State University); Bruno Alves Santarossa (Butantan Institute); Maria Cristina Motta (Federal University of Rio de Janeiro); Samuel Cota Teixeira (Federal University of Uberlândia); Eloisa Amalia Ferro (Federal University of Uberlândia); Simone Calderano (Butantan Institute); Miguel Angel Chiurrilo (University of Cincinnati); Noelia Lander (University of Cincinnati); Roberto Docampo (University of Georgia); Marcelo Santos da Silva (University of São Paulo)

Trypanosoma cruzi is the etiologic agent of Chagas disease, a frequently fatal illness that affects the heart and gastrointestinal systems. Available treatment options are only partially effective for the disease and can cause serious side effects on the patients. Thus, the search for the elucidation of molecular pathways that may provide potential targets for drug development is of paramount importance. Inositol pyrophosphates (PP-IPs) are involved in a wide range of processes in eukaryotes, such as pathogenicity, homologous recombination, and apoptosis signaling. These metabolites are synthesized by pathways involving the participation of IP6K kinase. However, the mechanism of action of PP-IPs in *T. cruzi* is poorly understood. Here, using the CRISPR/Cas9 approach, we disrupted a single allele of IP6K and analyzed the consequent phenotypic alterations. IP6K-deficient cells presented some morphological effects, such as rounding and wrinkling of the cell body, mitochondrial enlargement, and a slight increase in glycosomes. Notably, IP6K-/+ lineage presented a reduced differentiation capacity during metacyclogenesis, suggesting that this kinase is essential for metabolic alterations during *T. cruzi* life cycle. Interestingly, the IP6K-/+ lineage also presented a loss of infective potential (reduced invasion rate) within human cardiomyocytes. Furthermore, from 48 to 120 h after the invasion, the IP6K-deficient lineage showed impaired intracellular replication, with a predominant release of amastigote forms. Together, our findings suggest that PP-IPs are important for two fundamental processes in *T. cruzi*: cellular differentiation process and infection capacity, both possibly related to metabolic pathways generating an enigma about the role of PP-IPs in *T. cruzi*.

65 Key *Leishmania* trans-regulators are essential for parasite surveillance and infectivity

Ewan Parry (University of York); Natalia M.M. Teles (University of York); Rachel Neish (University of York); Adam Dowle (University of York); Katherine Newling (University of York); Eva Kyriakou (University of York); Fabiano Pais (University of York); Jeremy C. Mottram (University of York); Pegine B. Walrad (University of York)

Like other Kinetoplastids, gene expression in *Leishmania* species is overwhelmingly post-transcriptional. This elevates the importance of RNA binding proteins (RBPs) in these systems as the primary gene regulators. Building upon the *L. mexicana* RBPome we isolated previously (Pablos et al. MCP, 2019), 70 candidate trans-regulators were selected for further investigation. An *L. mexicana* barcoded trans-regulator knockout clone library was created using CRISPR-cas9 (Baker et al. Nat Comms, 2021) and screened through lifecycle progression to macrophage and mouse infections. Remarkably, 60% of the RBPs screened are essential for cell viability and 26% contribute to lifecycle progression to human-infectious stages, infectivity and/or virulence. Examination of individual knockout lines verify the screen outcomes of key RBPs essential for parasite growth, viability and infectivity. 13 RBPs were endogenously tagged, immunoprecipitated and submitted to transcriptomic and proteomic analyses to identify all RNP components. Specific trans-regulators associate with RNA pools enriched for different protein products. Protein interactome analyses reveal RBP clusters that may represent novel regulatory pathways critical for survival. Further analyses are underway to map interaction dynamics of these key regulators and the RNAs they regulate that drive differentiation and virulence capacity in *Leishmania*.

Poster Session A – Abstracts

Wednesday, September 11

7:00PM

8 (TT) Decoding single cell gene expression as parasites invade the adipose tissue

Lara López-Escobar (Instituto de Medicina Molecular); Milena Jakimovska Özdemir (Acibadem University); Ugur Sezerman (Acibadem University); Luisa M Figueiredo (Instituto de Medicina Molecular)

Trypanosoma brucei is a motile parasite that invades and adapts to organs, including adipose tissue. To explore parasite heterogeneity during mouse infection, we developed a 10x Genomics single-cell RNA sequencing (scRNAseq) protocol to capture parasites from blood and adipose tissue at the onset of tissue colonization. Our findings reveal that adipose tissue is initially colonized by slender forms in the G1 cell cycle stage. By day 5, these parasites proliferate and exhibit high glycolytic score similar to their bloodstream counterparts yet differ in 92 differentially expressed genes (logfc.threshold = 0.25). From days 6 to 7, some parasites differentiate into stumpy forms, which are cell cycle arrested and express stumpy-specific genes. Meanwhile, the slender population remains proliferative, but some start downregulating glycolytic enzymes and flagellum-associated proteins. These results confirm that functional tissue adaptation involves not only a metabolic shift, but it may also affect parasite shape and/or motility. VSG gene expression analysis revealed greater diversity in adipose tissue compared to blood, which is consistent with recent VSG-seq data. In conclusion, our data indicates that adipose-colonizing parasites are primarily slender forms in G1, which can differentiate into stumpy forms or adapt environmentally. We propose that these changes favor the survival of the parasite population, promoting emergence of VSG-switchers and ultimately contributing to disease chronicity.

14 (TT) Identifying molecular mediators of cellular states in *Trypanosoma cruzi*: comparison of single cell RNA sequencing technologies

Caroline D Keroack (Brown University); Sophie Marcus-Wade (Brown University); Jacqueline Lopez (Brown University); Eric D Salomaki (Brown University); Christopher L de Graffenried (Brown University)

Trypanosoma cruzi differentiates into various cellular states as it progresses through the insect host, shifting from the proliferative epimastigote to the infectious metacyclic trypomastigote. The cell cycle of chemically synchronized epimastigotes and the expression profiles of purified metacyclic trypomastigotes have been described using bulk RNA sequencing. These methods of synchronization or purification can be harsh to the cell and may alter gene expression. Single-cell RNA -sequencing is an ideal method for profiling the cell cycle and metacyclogenesis, as both are asynchronous in culture. Here, we demonstrate that *T. cruzi* polyadenylates ribosomal RNA, resulting in significant rRNA contamination in data generated using the 10X genomics pipeline, which relies on oligo(dT) capture. Deep sequencing can be employed to capture full transcriptomes from these samples, but this represents a significant financial burden and impacts the data quality. To address these issues, we have compared two scRNA-seq technologies – the microfluidic based 10X genomics platform and particle-templated instant partition sequencing (PIPseqTM, Fluent Biosciences) – to assess the relative rRNA contamination and compare the cluster markers identified. We show that PIPseq results in significantly fewer reads mapping to rRNA, resulting in higher coverage of transcripts of interest. Using these data, we have identified both conserved and *T. cruzi*-specific markers of cell cycle progression in *T. cruzi* epimastigotes. We also discovered marker genes of metacyclic trypomastigotes which further demonstrate significant reprogramming during metacyclogenesis. We are now focusing on using immunofluorescence microscopy and live cell imaging to validate candidate genes important to the different cellular states.

15 (TT) LeishGEM: A genome-scale database for knockout mutant life cycle fitness phenotyping and subcellular protein localisation in *Leishmania mexicana*

Ulrich Dobramysl (University of Oxford); Eden R Ferreira (University of York); Rachel P Neish (University of York); Laura D Davidson (Oxford Brookes University); Raquel Pereira (University of Bern); Ruth Etzensperger (University of Bern); Sidonie Aellig (University of Bern); Matthew Young (University of Glasgow); James Smith (University of Glasgow); Jeziel Damasceno (University of Glasgow); Jack D Sunter (Oxford Brookes University); Jeremy Mottram (University of York); Eva Gluenz (University of Bern); Richard Wheeler (University of Oxford)

The success of *Leishmania* parasites as pathogens is encoded in their genome. However, as eukaryotes, their genome is large and relatively complex. Despite extensive efforts for the functional characterisation of protein-coding genes in *Leishmania*, the role and localisation of most is still unclear. According to TriTrypDB only 14% of the 8,267 *L. mexicana* protein-coding genes have been unambiguously named, with the large majority remaining of putative function or hypothetical. The major aims of the *Leishmania* Genetic Modification (LeishGEM) project are to systematically address this by: 1) Determining the fitness of deletion mutants of protein-coding genes (genome-wide, 8,267 genes) by generating uniquely genetically barcoded deletion cell lines and assessing growth fitness as promastigotes, axenic amastigotes, amastigotes in macrophages, and in a mouse footpad infection. 2) Visualising the sub-cellular localisation of proteins in promastigotes and axenic amastigotes by tagging at both the N and C termini (if lacking an ortholog in or divergent from *T. brucei* - 2,700 target genes) in the LeishTag sub-project. 3) Analysing protein subcellular localisation via LOPIT-DC fractionation. The fitness phenotyping and localisation data generated by the LeishGEM project is available as we generate it at https://browse.leishgem.org/. As of June 2024, this database contains fitness data for 2,305 gene deletion mutants, subcellular localisation for 1,426 tagged cell lines where we have completed localisation annotation, and 3,782 proteins for which LOPIT-DC fractionation and mass spectroscopy yielded information on the subcellular localisation. This is the first data release of a transformative resource for the function of thousands of genes in a family of important human pathogens.

16 (TT) A novel conditional knockdown system in *T. cruzi* reveals essential roles for Aurora and Polo-like kinases in parasite cell division

Justin Wiedeman (University of Georgia); Ruby Harrison (University of Georgia); Drew Etheridge (University of Georgia)

The protozoan parasite *Trypanosoma cruzi* is responsible for Chagas disease, the most severe parasitic illness of the Americas. Despite the medical importance of *T. cruzi*, our knowledge of the identity and function of essential genes is severely limited by the lack of a conditional knockdown system. Unlike its more genetically tractable cousin *Trypanosoma brucei*, *T. cruzi* lacks the necessary enzymatic machinery to facilitate RNAi based functional studies. Additionally, the diploid nature of the *T. cruzi* genome and arrangement of genes into polycistronic "cassettes" which lack clearly defined promoters, precludes the use of standard molecular approaches to analyze essential gene function. Here we describe the development and use of a functional conditional knockdown system based on a tetracycline-responsive hammerhead ribozyme (HHR) inserted into the 3' un-translated (UTR) region of genes of interest. Using this new tool, we demonstrated the essential nature of multiple protein-coding genes, including the genes for the Aurora and Polo-like kinases. We discovered roles for these kinases in mitosis and cytokinesis, respectively, with these studies mirroring their known roles in *T. brucei*. Importantly, we demonstrated the effectiveness of the HHR knockdown system in intracellular amastigotes, the medically relevant mammalian stage of the parasite. This conditional knockdown system allows, for the first time, the straightforward functional characterization of essential genes in *T. cruzi*.

17 (TT) A 2A peptide-based epitope-tagging toolkit for analysis of protein localisation, regulation, and function in Trypanosomatids

Carla Gilabert Carbajo (Imperial College London); Xiaoyang Han (Imperial College London); Bhairavi Savur (Imperial College London); Arushi Upadhyaya (Imperial College London); Fatima Taha (Imperial College London); Richard J Wheeler (University of Oxford); Michele Tinti (University of Dundee); Phillip Yates (Oregon Health and Science University); Calvin Tiengwe (Imperial College London)

Kinetoplastid parasites cause serious diseases affecting human and animal health. As these parasites transition between vertebrate hosts and insect vectors, their survival relies on precise gene expression regulation to adapt to environmental changes such as temperature and nutrient availability. Since gene regulation in Kinetoplastids is primarily post-transcriptional, developing an efficient gene tagging tool that retains important cis-regulatory elements is crucial for investigating protein localisation and function under specific conditions. Here, we present a new tagging system that preserves cis-acting gene regulatory elements. Our system combines CRISPR/Cas9-mediated editing with the 2A peptide-based system to achieve bicistronic expression of a drug-selectable marker and a tagged gene from its endogenous locus. The 2A peptide sequence causes a “ribosomal skip” event during translation, allowing the single bicistronic transcript to produce separate functional proteins. We demonstrate the functionality of our system by tagging six *Trypanosoma brucei* proteins with mNeonGreen or mScarlet in different cellular compartments: ESAG3 (ER), cytosolic Hsp70, a subunit of the iron transporter the transferrin receptor (ESAG7), two cytoskeletal calpain-related proteins (CAP5.5, CAP5.5V), and glycosylphosphatidylinositol phospholipase C (GPI-PLC). We show: (i) high-efficiency separation of drug marker and target 2A-modified protein modified, (ii) >90% positive selection of all transgenic cell lines, (iii) differential regulation of Hsp70 to heat shock, (iv) iron-dependent regulation of ESAG7 and functionality by transferrin endocytosis, and (v) stage-specific developmental regulation of CAP5.5, CAP5.5V, and GPI-PLC during in vitro differentiation. Our 2A-based tagging toolkit is applicable to all kinetoplastids amenable to CRISPR/Cas9 gene editing. It is useful for studying post-transcriptional and post-translational regulation within a single experiment.

25 (TT) The hydrophobic effect drives the outer-to-inner mitochondrial membrane connection of the Trypanosomal tripartite attachment complex

Philip Stettler (Univeristy of Bern); Salome Aeschlimann (Univeristy of Bern); Bernd Schimanski (Univeristy of Bern); Sandro Käser (Univeristy of Bern); André Schneider (Univeristy of Bern)

Genome inheritance is essential for all lifeforms. Trypanosomes are single celled organisms harboring a single mitochondrion that use a unique protein complex, the tripartite attachment complex (TAC), to segregate their mitochondrial DNA. The TAC links the mitochondrial DNA network to the flagellum, ensuring its proper segregation during cytokinesis. This study focuses on a key connection within the TAC between two of its subunits: TAC60 and p166. TAC60 is a protein of the outer mitochondrial membrane and interacts with p166, a membrane protein of the inner mitochondrial membrane. This interaction creates a unique mitochondrial membrane contact site. Here, we have investigated this interaction using an AlphaFold structure model as well as an in vitro peptide-protein interaction screen. This resulted in the discovery of a well conserved kinked α-helix in TAC60 at the binding site of p166. In vivo experiments revealed that the tertiary structure of this α-helix is crucial for its interaction with p166. Surprisingly, some highly conserved charged amino acids are dispensable for this interaction. Instead, less well conserved hydrophobic amino acids in the interaction motifs of both TAC60 and p166 mediate the interaction which is therefor a result of the 'hydrophobic effect'. Consequently, the protein interaction is very stable in vivo and displays a high tolerance to increased salt concentrations in vitro. In summary, we characterized the central interaction connecting the cytosolic and the inner module of the Trypanosomal TAC. This is the first study to investigate the integrity of the TAC on a sub-protein level.

26 (TT) Bicarbonate – CO2 sensing and response mediated by trypanosome QIQ1

Anna Trenaman (University of Dundee); Federico Rojas (University of Edinburgh); Michele Tinti (University of Dundee); Keith Matthews (University of Edinburgh); Sam Alsford (London School of Hygiene and Tropical Medicine); David Horn (University of Dundee)

African trypanosomes occupy distinct environments in their mammalian host, and during their life cycle, and adapt their metabolism accordingly. A quorum-sensing pathway has been described in bloodstream-form trypanosomes, for example, involving oligopeptide-sensing and differentiation, but the pathways involved in sensing and responding to other environmental cues remain incompletely characterised. We now describe a bicarbonate-sensing mechanism. A genome-scale loss-of-function genetic screen revealed knockdowns associated with gain-of-fitness, those that increase trypanosome growth. The dominant hit (Tb927.8.6870) encodes a flagellum-localised protein with multiple putative calmodulin binding IQ-domains. We name this protein QIQ1, for Quintuple IQ-domain protein 1, also reflecting the 'quick' growth phenotype observed following knockdown. qiq1-null trypanosomes display a growth advantage both in vitro and in vivo; in culture and in a mouse model, respectively. These mutants maintained morphological differentiation and expression of a quorum-sensing marker in vivo indicating placement of QIQ1 in a distinct pathway. The IQ domains suggested a calcium-signalling mechanism, and consistent with this view, calcium chelators abolished the competitive advantage displayed by qiq1-null cells. We tested several potential environmental cues and found that the competitive advantage of qiq1-null cells was abolished in the absence of sodium bicarbonate. Proteomic analysis revealed a deficit in the modulation of mitochondrial ATP-synthase and citric acid cycle enzymes in the absence of QIQ1, while preliminary metabolomic analysis revealed specific shifts in ATP levels, and in other nucleotides and amino acids, consistent with metabolic remodelling. We conclude that trypanosomes sense bicarbonate in a QIQ1- and calcium-dependent manner and elicit a metabolic response. This response to an external stimulus may facilitate adaptation to resource-limiting environments.

27 (TT) A genome-wide overexpression screen identifies proteins that inhibits the growth of *Trypanosoma brucei*

Navina Panneer Selvan (Rutgers University); Aditi Mukherjee (Rutgers University); Esteban Erben (IIBIO-UNSAM); Hee-Sook Kim (Rutgers University)

Trypanosoma brucei is a protozoan parasite that causes African trypanosomiasis in humans and animals in sub-Saharan Africa. The disease is fatal if left untreated and proceeds from a peripheral to a central nervous system (CNS) infection. Development of vaccines have been hampered due to the surface antigen switching occurring in *T. brucei* cells, which allows the parasite to escape the host immune recognition. Genome-wide screening tools can facilitate the identification of potential drug target proteins and pathways. Gene depletion method using RNAi library has been used for such studies in *T. brucei*. To develop a complementary gain-of-function tool, we generated *T. brucei* overexpression (OE) libraries and validated them in drug resistance screening (Carter et al, 2020), which discovered proteins that confer resistance to melarsoprol (a drug for stage II HAT). Overexpression of some genes can inhibit cell proliferation, while deletion of them has no effect. To identify 'toxic' genes that inhibit the growth of *T. brucei* cells when they are overexpressed, we screened ~400,000 OE library transfected trypanosome cells and isolated about 200 'toxic' genes using a targeted high-throughput DNA-seq. We validated three candidates (HYP1, HYP2, and HYP3) for *T. brucei* cell growth inhibition. Through RNA-seq analysis, we discovered that HYP1 and HYP2 are involved in transcriptional silencing and HYP3 is important for cell-cycle regulation. We will discuss how the overexpression of these proteins might affect the growth of *T. brucei*.

28 (TT) Host-pathogen dynamics of bumble bee parasite, *Crithidia bombi*

Blyssalyn V Bieber (Villanova University); Faith St. Clair (Villanova University); Sarah Lockett (Villanova University); Sonja Glasser (University of Massachusetts Amherst); Lynn S Adler (University of Massachusetts Amherst); Megan L Povelones (Villanova University)

Crithidia bombi is a monoxenous Trypanosomatid that infects bumble bees by attaching to their intestinal tract, impacting survival of workers and overwintering queens. There is extensive research on the ecology of this host-pathogen system, however, less is known about the cellular mechanisms mediating infection dynamics. Researchers typically maintain *C. bombi* in laboratory-reared bumble bee (Bombus impatiens) colonies, but parasites can also be culture adapted. We obtained 30 clonal isolates from laboratory B. impatiens and are examining their phenotypic and molecular diversity, including sequencing to determine haplotypes. This data will be compared to other field isolates providing a baseline for studies on host specificity and determinants of infection. We modified one isolate to express either GFP or RFP and used these parasites to infect B. impatiens, allowing for in vivo visualization. By expanding the genetic toolkit for *C. bombi*, we will better understand factors that affect parasite survival and host colonization. For example, sunflower (Helianthus annuus) pollen has been shown to prevent and reduce *C. bombi* infections in bumble bees. We explored this interaction in vitro by applying sunflower pollen to cultured *C. bombi* parasites expressing a nano-Luciferase construct. While growth was unaffected by pollen exines derived from either buckwheat or sunflower, sunflower pollen specifically disrupted attachment of parasites to tissue culture plates. Furthermore, sunflower exines damage or remove attached *C. bombi* growing as rosettes. We are continuing to explore this interaction to better understand how sunflower exines might act on parasites in vivo to reduce pathogen transmission in natural settings.

67 A parasite's life cycle of RNA methylation

Lúcia Serra (Instituto de Medicina Molecular); Sara Silva-Pereira (Católica Biomedical Research Centre); Idálio J. Viegas (Institute of Inflammation and Ageing); Henrique Machado (Instituto de Medicina Molecular); Lara López-Escobar (Instituto de Medicina Molecular); Luisa M Figueiredo (Instituto de Medicina Molecular)

N6-methyladenosine (m6A) is the most abundant mRNA modification in eukaryotes, with important roles in gene expression. In African trypanosomes, this post-transcriptional modification is detected in hundreds of transcripts and it affects the stability of the variant surface glycoprotein (VSG) transcript in the proliferating bloodstream form. However, how m6A landscape varies across the life cycle remains poorly defined. Using full-length, non-fragmented RNA, we immunoprecipitated and sequenced m6A-modified transcripts across three life cycle stages of *Trypanosoma brucei* – slender (proliferative), stumpy (quiescent), and procyclic forms (proliferative). We found that 1037 transcripts are methylated in at least one of these three life cycle stages. While 21% of methylated transcripts are common in the three stages of the life cycle, globally each stage has a distinct methylome. Interestingly, 47% of methylated transcripts are detected in the quiescent stumpy form only, suggesting a critical role for m6A when parasites exit the cell cycle and prepare for transmission by the tsetse fly. In this stage, we found that a significant proportion of methylated transcripts encodes for proteins involved in RNA metabolism, which is consistent with their reduced transcription and translation. Moreover, we found that within the VSG repertoire, not all VSG transcripts are demethylated upon parasite differentiation to procyclic form. Our study reveals that the m6A regulatory landscape is specific to each life cycle stage, becoming more pervasive as *T. brucei* exits the cell cycle.

68 Characterization of a universally conserved histidine critical for tRNA deaminase activity

Aubree A Zimmer (Brown University, The Ohio State University); Luciano G Dolce (EMBL); Eva Kowalinski (EMBL); Diana Lopez (The Ohio State University); Marcos Sotomayor (The Ohio State University); Juan Alfonzo (Brown University)

Transfer RNAs (tRNAs) are central to protein synthesis by converting genomic information of protein-coding regions into a precise polypeptide chain, as dictated by codons in mRNA. tRNAs require extensive chemical modifications for structural stability, translation fidelity, as well as other decoding functions. One such modification is the deamination of adenosine to inosine at the first position of the anticodon, which is essential for viability in bacteria and eukaryotes. Inosine formation at this wobble position in eukaryotes increases the decoding capabilities of a tRNA to recognize multiple different synonymous codons. ADAT2/3, the deaminase responsible for A-to-I activity in eukaryotes, including *Trypanosoma brucei*, has evolved to deaminate 7-8 tRNAs independent of sequence specificity. How the eukaryotic deaminase recognizes its multiple substrates from other RNAs is still unclear. In collaboration with the Kowalinski laboratory at the EMBL, we determined multiple tRNA recognition sites using the first structure of a eukaryotic tRNA deaminase (*Trypanosoma brucei* ADAT2/3) bound to substrate. Sequence alignments revealed a previously uncharacterized but universally conserved histidine near the enzyme's active site. Using biochemical assays and molecular dynamics simulations, we show the importance of the conserved histine for enzymatic activity and provide insight into a potentially new scaffold for tRNA deaminases.

69 Using an undergraduate research course to study of *Trypanosoma brucei* malate dehydrogenase isoforms using phosphomimetic mutations

Amy L Springer (University of Massachusetts, Amherst); Joseph J Provost (University of San Diego)

Performing scientific research as an undergraduate is associated with higher persistence in STEM majors and greater sense of scientific independence. Here we describe using Course-based Research Experiences (CUREs) to provide opportunities for undergraduate research in their classroom on parasitology projects. In this protein-based CURE, phosphomimetic mutations of Malate Dehydrogenase (MDH) from *Trypanosoma brucei* were used to study functions of different MDH regions. MDH is a highly conserved enzyme that catalyzes NAD-dependent interconversion of oxaloacetate and malate. It plays important roles in redox balance and provides intermediates for catabolic and anabolic pathways. *T. brucei* has three functional MDH isoforms localized, respectively, to mitochondria, cytoplasm and glycosome. Comparative structural and functional information from MDH of model organisms was used to predict putative phosphorylation sites in each of the *T. brucei* MDH isoforms. Students designed mutations, made predictions about their effects and then expressed and characterized the mutant proteins. Results from T192D (cytoplasmic MDH) and T154D (mitochondrial MDH) show reduced specific activity and changes in pH profile. During the project, students work with an external MDH collaborator to discuss their hypotheses and conduct their experiments which provides a sense of the greater scientific community through MDH CUREs Community (MCC), an NSF-funded network of faculty supporting each other to teach MDH CUREs that offers resources, mentoring and collaborations to support protein-based CUREs. In assessments, students reported the conversations with the collaborator helped them to focus and develop ideas about their projects, and faculty reported increased faculty satisfaction with teaching CUREs.

70 Glycosomal Fission in *Trypanosoma brucei*: The Critical Roles of TbDLP and TbFis1

Advaitha Iyer (DCBP, University of Bern); Silke Oeljeklaus (Biozentrum, University of Würzburg); Bettina Warscheid (Biozentrum, University of Würzburg); André Schneider (DCBP, University of Bern)

The Dynamin-like protein (DLP) is a integral component of mitochondrial and peroxisomal fission machinery in yeast and mammalian cells alike. While its role in mitochondrial fission has been extensively studied in trypanosomes, its involvement in glycosomal (Trypanosomal peroxisome equivalent) fission remains unclear. This study aims to elucidate whether the typically shared mitochondrial and peroxisomal fission machinery exists in trypanosomes and to understand how individual proteins of this machinery contribute to glycosomal fission in the parasite. Our findings demonstrate that both TbDLP and TbFission1 (Fis1, a well-known peroxisomal fission factor in yeast and mammalian cells) are integral to the glycosomal fission machinery in trypanosomes. Knockdown of TbDLP, an essential protein in the parasite, results in a dramatic glycosomal 'bursting' phenotype, visualised using transmission electron microscopy. Interestingly, TbFis1 knockout parasites exhibit highly 'distended' glycosomes as well as glycosomal 'budding'. This study is the first to show that the absence of either TbDLP or TbFis1 in trypanosomes leads to a disruption in glycosomal fission, with markedly different outcomes for each protein. The glycosomal 'bursting' phenotype observed in TbDLP knockdown suggests a critical role for TbDLP in maintaining glycosomal integrity. In contrast, the 'distended' and 'budding' phenotypes in TbFis1 knockout parasites indicate a distinct yet equally important role for TbFis1 in glycosomal fission. These findings suggest unique roles for both TbDLP and TbFis1 in maintaining glycosomal fission and highlight the complexity of the fission machinery in trypanosomes, providing new insights into the unique aspects of parasite cell biology.

71 Dynamic Regulation of LmCOX4 and Metabolic Adaptation in *Leishmania* Under Mammalian Heat Stress

Isabel Stephany Brassesco (LSU Health New Orleans); Edward Wojcik (LSU Health New Orleans); Ben Kelly (LSU Health New Orleans)

During their infectious life cycle, *Leishmania* parasites must rapidly adapt to significant environmental shifts, such as a sudden increase in temperature when transitioning from the sandfly vector to the mammalian host. Our prior studies demonstrated that robust expression of LmCOX4, a nuclear-encoded mitochondrial protein integral to Complex IV of the electron transport chain (ETC), is crucial for optimal mitochondrial fitness and replication in macrophages. Unexpectedly, upon exposure to mammalian temperatures (~35°C), *Leishmania major* promastigotes exhibit a transient downregulation of LmCOX4 expression. To elucidate this phenomenon, we hypothesize that mammalian temperature induces mitochondrial stress, leading to the degradation of LmCOX4 and a consequent partial reduction in mitochondrial membrane potential (MMP). As MMP is essential for driving mitochondrial protein import, its reduction may result in the mis-localization and subsequent homeostatic degradation of nascent LmCOX4, thus diminishing Complex IV activity and further lowering MMP to a basal level. We propose that the induction of mitochondrial heat shock proteins in response to 35°C facilitates a gradual re-accumulation of mitochondrial LmCOX4 through ongoing low-level import driven by basal MMP. This process ultimately restores MMP and LmCOX4 levels, thereby re-establishing mitochondrial function at 35°C. To test our hypothesis, we are examining whether the mitochondrial targeting sequence of LmCOX4 is less functional at 35°C compared to 27°C. Additionally, we are assessing the biological consequences of transient LmCOX4 loss by comparing mitochondrial versus glycolytic ATP production at 35°C using a Seahorse instrument.

72 Dynamics within the *Trypanosoma brucei* subpellicular microtubule array

Laura A Smithson (Brown University); Kevin E Ramrattan (Brown University); Emily K Campbell (Brown University); August Guang (Brown University); Christopher L de Graffenried (Brown University)

The cytoskeleton is an essential component of eukaryotic organisms, having evolved to facilitate the occupation of distinct biological niches. Microtubules can provide structural organization to cells through their assembly into large, complex structures. *Trypanosoma brucei* relies on a highly crosslinked network of subpellicular microtubules underlying the plasma membrane to regulate cell morphogenesis. This subpellicular microtubule array (SPA) undergoes constant dynamic change through the turnover of microtubules in a highly regulated process that is mostly uncharacterized. Despite the central role of the SPA in kinetoplastid morphogenesis, very little is known about the molecular components that are involved in building and organizing this structure. Visualization the SPA has traditionally relied on electron microscopy which can be limited by slice thickness, cellular helicity and volume. Recently, we have optimized protocols for ultrastructure expansion microscopy (uExM) coupled with super-resolution light microscopy that has allowed for visualization of microtubule organization at a resolution approaching that of electron microscopy. Using this strategy, essential microtubule structures such as microtubule nucleation and termination sites, as well as the microtubule crosslinks can be imaged at high resolution. Furthermore, we have localized several microtubule-associated proteins (MAPs) to SPA subdomains, including PAVE1. We show that the microtubule crosslinks are indeed asymmetrical, with PAVE1 and two other MAPs localized exclusively to opposite sides of the crosslinks. Additionally, we are developing a novel pipeline for automated 3-dimensonal reconstruction of whole microtubule arrays using Machine Learning and Serial Block-Face Scanning Electron Microscopy (SBF-SEM). This allows us to remove the constraints of manual microtubule tracking methods and will provide a robust and much needed tool for examining large populations of cells at high resolution.

73 Investigating modulation of translation initiation in *Leishmania* parasites

Diana Calvopina Chavez (Harvard Medical School); Melissa Leger-Abraham (Harvard Medical School)

Leishmania parasites are the causative agents of visceral, cutaneous, and mucocutaneous Leishmaniasis, and pose a significant global health burden with 700,000 to 1 million new cases reported annually. *Leishmania* parasites reside in the midgut of female sandflies as flagellated promastigotes. Following a blood meal, infected sandflies transmit these parasites to hosts (e.g., humans), where they live inside macrophages as obligate intracellular amastigotes. The ability of *Leishmania* to adapt to these two very different environments requires rapid reprogramming of gene expression, which is predominantly regulated at the translation initiation level. Understanding how *Leishmania* controls protein levels could provide critical insights for developing targeted translation initiation inhibitors against these parasites. In this study, we investigate a unique protein-protein interaction between the cap-binding protein LIF4E-1 and the multi-subunit complex LIF3, crucial for mRNA recruitment to the small ribosomal subunit in the human stage. Our hypothesis posits that LIF4E-1 interacts with one subunit of LIF3, and this interaction plays a pivotal role in facilitating pre-initiation complex (PIC) assembly in amastigotes, bypassing the need for eIF4G-like scaffolding proteins. To test this, we use biochemical, biophysical, and structural approaches to characterize the LIF4E-1/LIF3 interaction. Through pull-down assays and Mass Spectrometry analysis, we determined a fragment of one of the subunit of LIF3 that interacts with LIF4E-1. Detailed structural analyses will determine the minimal binding epitope that could inform the development of drugs targeting this crucial LIF4E-1/LIF3 interaction.

74 MPsome-RNA Helicase Nexus Governs Mitochondrial RNA Biogenesis in Trypanosomes

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Discoveries of terminal RNA uridyltransferases (TUTases) in mitochondria of trypanosomes positioned 3’ uridylation as the major epitranscriptomic factor. We have identified the mitochondrial 3’ processome (MPsome) composed of KRET1 TUTase, KDSS1 3’-5’ exonuclease, and five 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, some of which involve double-stranded RNA intermediates arising from cryptic antisense molecules. These duplexes block MPsome’s progression along a precursor thus generating mature RNAs. Participation of an unknown factor in resolving these double-stranded intermediates has been hypothesized but its identity remained unknown. Here, we report a previously uncharacterized DEAH-box RNA helicase, termed KREH3, that fulfils this key mechanistic step. Upon the MPsome stalling near the duplex region, KREH3 unwinds the duplex, and binds to and stabilizes properly trimmed gRNAs. We demonstrate that ATP-dependent unwinding activity of KREH3 is essential for processing rRNA, mRNA and gRNA precursors. We further show that KRET1, KDSS1, and KREH3 co-localize in antipodal sites 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 kinetoplast RNA processing and reveal that spatially and functionally coupled TUTase, 3’-5’ exonuclease and ATP-dependent RNA helicase activities underpin mitochondrial RNA biogenesis in trypanosomes.

75 Polyadenylation of ribosomal RNA by *Trypanosoma cruzi* and *Trypanosoma brucei* complicates use of RNA sequencing technology

Caroline D Keroack (Brown University); Eric Salomaki (Brown University); Christopher L deGraffenried (Brown University)

Kinetoplastids are a highly divergent lineage of eukaryotes. Their unique biology presents challenges when using methodologies optimized for model eukaryotic organisms. Typically, eukaryotic cells strictly process their mRNA, giving it a 5' cap and poly(A)-tail to facilitate increased stability and translation into protein. In general, other varieties of untranslated RNA are not polyadenylated. It has been shown that *Leishmania* polyadenylates its ribosomal RNA (rRNA). This poses complications when studying the gene expression of these parasites because many RNA sequencing technologies rely on oligo(dT) capture for library generation. Previously, it was unknown if other kinetoplastids also polyadenylated ribosomal RNA. We have performed single-cell RNA sequencing in *Trypanosoma cruzi* and found that a significant proportion of the data aligned to ribosomal RNA–the majority aligning to the 18S rRNA locus, suggesting that rRNA is polyadenylated in this parasite. This greatly impacts our ability to accurately represent the transcriptome using oligo(dT) capture techniques. Due to this result, we sought to demonstrate conclusively that both *Trypanosoma cruzi* and *Trypanosoma brucei* polyadenylate their ribosomal RNA. We used PCR to show that *T. cruzi* and *T. brucei* both polyadenylate the 18S rRNA transcripts. We have also shown that a substantial proportion of the 18S rRNA is polyadenylated compared to total 18S rRNA transcripts via qPCR. This phenomenon is not strain specific, as *T. cruzi* Y strain and Brazil A4 both polyadenylate their rRNA. These findings have implications for experimental design for the Trypanosomatids and highlight distinctive eukaryotic biology with regard to RNA processing.

76 Development of an Inducible Protein Degron System in *Trypanosoma brucei* and *Trypanosoma cruzi* to Facilitate Study of Essential Genes

Jacqueline Lopez (Brown University); Caroline Keroack (Brown University); Sophie Marcus-Wade (Brown University); Christopher L de Graffenried (Brown University)

Trypanosomatids are flagellated protist parasites responsible for insect-borne diseases that are serious neglected public health problems worldwide. The study of essential genes in these organisms is a priority for discovering new drug targets and vaccine development. Research in *T. brucei* has benefited from the use of RNA interference (RNAi), allowing for inducible knockdown of essential genes. However, the lack of RNAi and inducible knockdown techniques generally in *T. cruzi* has made it challenging for researchers to investigate essential genes. To overcome this limitation, we have adapted an inducible protein degron system known as the SMASh-tag to *T. brucei* and *T. cruzi*. In this system, the gene of interest is tagged with a viral protease and linked to a degron domain. In the untreated condition, the modified protein self cleaves, releasing the degron and producing the essentially wild-type protein. When an inhibitor that is highly specific for the viral protease is added, the process of self-cleavage is blocked, causing the degradation of the tagged protein via the degron domain. We are testing the efficacy of both N-terminal and C-terminal SMASh-tag using YFP as a substrate. We show that this system is effective at producing inducible knock down (~70%) in genome-integrated C-terminally tagged YFP. The N-terminal SMASh-tag however does not function properly in *T. brucei*: the protease portion of the system is functional, but the degron-tagged protein accumulates after protease inhibition rather than being degraded. Our current efforts focus on employing the C-terminal SMASh-tag to inducibly knock down essential proteins.

77 Uncovering links between codon bias and tRNA expression dynamics in *Trypanosoma cruzi* life forms

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Codon usage bias, characterized by the preferential selection of synonymous codons, impacts expression of mRNA and protein across all kingdoms of life, including Trypanosomatids. These unicellular parasites, such as *Trypanosoma cruzi*, the cause of Chagas disease, primarily regulate their protein-coding genes through post-transcriptional mechanisms. Here, we combined analyses of codon usage with multiple high-throughput sequencing data to investigate how codon usage optimizes the production of proteins in the developmental stages of *T. cruzi*. tRNA sequencing was employed for the first time in Trypanosomatids, revealing co-adaptation between codon usage and anticodon availability. Surprisingly, despite notable differences in the transcriptomes and proteomes of metacyclic trypomastigotes (MT) and epimastigotes (EPI), these two *T. cruzi* developmental stages exhibited similar pools of tRNAs and similar codon usage preferences. Moreover, we observed a strong correlation between the open chromatin levels of tRNA genes with tRNA expression in EPI but not in MT, suggesting that the tRNA pool in the MT might be derived from the EPI. Additionally, our analysis unveiled a relationship between anticodon:codon pairing modes and mRNA abundance. Highly expressed mRNAs favored Watson-Crick base pairing, whereas less expressed mRNAs displayed more wobble base pairing. Overall, our findings suggest that protein expression in *T. cruzi* EPI and MT may be influenced by a combination of codon usage bias, tRNA abundance, and anticodon:codon pairing modes. This study sheds light on the intricate mechanisms governing gene expression in *T. cruzi* and provides insights into its differentiation and pathogenicity.

78 Direct demonstration that specific histone H4 tail lysines impact chromatin-based mechanisms in trypanosomes

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

It remains unclear to what extent the control of transcription, DNA replication and DNA repair rely upon chromatin-based mechanisms in Trypanosomatids. The histone H4 N-terminal tail, and tail lysine (K) acetylation, play important roles in these processes in other eukaryotes, for example, but the Trypanosomatid histone H4 tail is highly divergent relative to the usual model eukaryotes. Since interpretation of 'writer', 'reader' and 'eraser'-defective phenotypes is complicated by potential perturbation of multiple histone substrates, and of non-histone substrates, we sought to entirely replace native *T. brucei* histone H4 with bespoke tail-edited versions of histone H4. We used an inducible CRISPR/Cas9 system to delete >40 native copies of the histone H4 genes, complementing the defect with a single, recoded and highly expressed ectopic copy. Templated codon editing was then used for site saturation mutagenesis of N-terminal tail residues in the ectopic H4 gene, including K4, K10 and K14. Fitness profiling, using multiplex amplicon-sequencing, revealed many edits that were tolerated at the K4 or K14 positions, while stop-codons and non-synonymous K10 edits were not tolerated. A panel of strains exclusively expressing novel histone H4-K mutants, including arginine (R; non-acetylated mimic) or glutamine (Q; constitutively acetylated mimic) substitutions, or K4 deletion, was then assembled and assessed. K4 mutants were hypersensitive to DNA damage, while both transcriptomic and proteomic analysis revealed moderate, but specific, perturbation of Variant Surface Glycoprotein gene silencing in these cells, providing the first direct evidence that histone tail residues and their modifications have specific impacts on DNA repair and gene expression controls in trypanosomes. K4R/K5R double mutants may display a more pronounced DNA repair defect, and we are currently testing this hypothesis.

79 Molecular dissection of VSG-Exclusion Protein 2 function and recruitment to the expression-site body

Sarah Stevens (University of York); Kiera Hayton (University of York); Josh Van Der Merwe (University of York); Beth Spink (University of York); Joana R Correia Faria (University of York)

African trypanosomes are masters of disguise! Stochastic antigenic switching allows these parasites to evade the host immune response. They possess an extensive repertoire of genes encoding for variant-surface-glycoproteins (VSGs), but only one is active at a time. The single active-VSG is transcribed by Pol-I within the expression-site-body (ESB). VSG-Exclusion-2 (VEX2) specifically localises to the ESB in bloodstream forms and has been shown to somehow sustain VSG singular expression. Here, we sought to dissect VEX2 function and recruitment to the ESB. Firstly, VEX2 full-length and VEX2-N overexpression leads to its accumulation at both the ESB and the nucleolar periphery, revealing intrinsic 'affinity' for sites of Pol-I transcription, but preferential localisation to the ESB. Secondly, specifically blocking transcription at the active-VSG-ES using a TetON/TetOFF system results in VEX2 mislocalisation and multiple VSG expression (RNA-Seq) indicating a role for RNA in its compartmentalisation. Thirdly, we found that VEX2 is SUMOylated and perturbations of SUMO metabolism, either by depletion of SUMO itself or depletion of a SUMO E3 ligase found in proximity to VEX2 (TurboID LC-MS/MS), renders VEX2 nucleolar or nucleoplasm disperse. Moreover, in mutants incapable of polySUMOylation, VEX2 localises to both the ESB and the nucleolus. Combined, these observations demonstrate that VEX2 recruitment to the ESB depends on sequences within its N-terminus, RNA and polySUMOylation. Lastly, VEX2 is a large RNA:DNA helicase. Precision editing using CRISPR/Cas9 showed that its helicase activity is required for VSG expression control, and DRIP-Seq before and after VEX2 depletion suggests a role in R-Loop metabolism at the active-VSG-ES.

80 A role for VSG mRNA in driving allelic competition

Douglas O Escrivani (University of Dundee); Michele Tinti (University of Dundee); Jane Wright (University of Dundee); Sebastian Hutchinson (Institut Pasteur, Paris); Anna Trenaman (University of Dundee); Joana R C Faria (University of York); David Horn (University of Dundee)

Among sixteen promoter-associated, and telomeric, Variant Surface Glycoprotein (VSG) genes in each bloodstream-form African trypanosome (427 strain), one produces the most abundant cellular mRNA and protein, while the others produce ~10,000-fold less mRNA (and protein). This extreme form of allelic competition and transcriptional dominance underpins antigenic variation and immune evasion. Several VSG regulatory proteins have been identified and characterised, representing substantial advances in our understanding, but what remains unclear is how these proteins (alongside other factors) enforce monogenic expression. We conducted a meta-analysis of >20 published RNA-seq datasets, which highlighted the roles of known VSG positive regulators (ESB1 and CFB2), telomere binding proteins (RAP1, PIP5Pase), and VSG exclusion factors (VEX1-2). We have also further explored the role of VSG mRNA. Neither knockdown of the active VSG transcript using RNA interference, or blocking translation of the active transcript, using the MS2 protein, substantially impacted VSG exclusion. In contrast, and consistent with a role for chromatin in reinforcing the exclusion of native VSGs, a transfected VSG cassette could escape exclusion, particularly when delivered under transient VSG-knockdown. The results suggest that establishment of monogenic VSG transcription can be driven by competing VSG transcripts, possibly based on competition for binding and sequestration of chromatin-associated RNA-binding proteins, such as RAP1 and VEX2. We, therefore, propose a non-coding function for VSG transcripts in driving allelic competition and transcriptional dominance.

81 Characterization of TbTRF's TERRA binding and telomeric dsDNA binding activities

Lovlesh Thakur (Cleveland State University); Bibo Li (Cleveland State University)

Trypanosoma brucei, the causative agent of human African trypanosomiasis, proliferates in the extracellular spaces inside its mammalian host, expresses VSG as its major surface antigen, and evades the host's immune response through VSG switching. VSG genes are located at subtelomeres and transcribed by RNA polymerase I in a strictly monoallelic manner. We have shown that binding of TbTRF, a telomere protein, to the duplex telomeric DNA is essential for proper maintenance of the telomere integrity and stability. Specifically, we have demonstrated that depletion of TbTRF results in a higher level of telomere repeat containing RNA (TERRA), more telomeric R-loops (TRLs), more telomeric DNA double-strand breaks (DSBs), and more recombination-mediated VSG switching events. TbTRF depletion reduces the number of nuclear TERRA foci, suggesting that TbTRF facilitates trans-localization of TERRA from its transcription site. Additionally, TbTRF binds both TERRA and duplex telomeric DNA simultaneously. We hypothesize that TbTRF's binding to TERRA is critical for suppressing TERRA and TRL levels. Based on the NMR structure of the TbTRF myb domain, we did RNABindRPlus analysis and predicted that several residues located on TbTRF myb helices may directly interact with TERRA. We introduced single and double mutations in the TbTRF myb domain, expressed the recombinant fragments, and examined their TERRA and telomeric dsDNA binding activities by electrophoretic mobility shift assay (EMSA). Furthermore, to investigate phenotypes of TbTRF mutations in-vivo, we are establishing a strain in which one endogenous TbTRF allele is flanked by loxP repeats, enabling its conditional deletion by Cre-mediated recombination. Our investigations will help reveal underlying mechanisms how TbTRF regulates TERRA and TRL levels.

82 de novo genome assembly of *Trypanosoma brucei* gambiense type 2 (Tbg2) and analysis of *T. brucei* subspecies population genetic structure

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Hybridisation has been shown to dramatically impact the evolution of pathogenic protists by altering biological potential and/or the acquisition of drug resistance. African trypanosomes are protists capable of causing severe disease in humans and animals across sub-Saharan Africa. Laboratory crosses in tsetse flies, the vector for both human and animal infective forms of the parasite, have demonstrated the capacity to generate hybrid species, but the extent to which this is occurring in nature remains unreported and controversial. Our analysis of 109 isolates at WGS resolution identified extensive incongruence among isolates that shared similar mitochondrial genomes but highly divergent nuclear genomes, identifying an unexpectedly high rate of inter-specific hybrids as the most parsimonious explanation. Interbreeding appeared to be highest among isolates designated *Trypanosoma brucei* gambiense type 2 (Tbg2). However, no reference genome for Tbg2 currently exists. To facilitate our genome-wide recombination analyses, we integrated PacBio and MinION long-read sequencing technologies to generate a de novo assembly of the Tbg2 isolate STIB 386. We have constructed a chromosome-level genomic assembly with an N50 of 3190.5Kb across 79 contigs ranging from 6204Kb to 25Kb, totalling 49.8Mbp which is bigger than the reference genome but consistent with recent assemblies. We have also annotated approximately 13,000 genes. This new assembly will aid in detecting recombinant strains, understanding trypanosome population dynamics, and supporting disease control strategies, and serve as a valuable asset for trypanosome research.

83 Trypanosomatids and EdU: a good example of dose-response relationship

Marcos Antonio F de Oliveira (University of São Paulo); Vitor Luiz da Silva (University of São Paulo); Thaise L Teixeira (University of São Paulo); Bryan E Abuchery (University of São Paulo); Giovana Z Luduvice (University of São Paulo); Fernanda M Gerolamo (University of São Paulo); Marcelo S da Silva (University of São Paulo)

EdU (5-Ethynyl-2'-deoxyuridine) and BrdU (5-bromo-2'-deoxyuridine) are thymidine analogs used to monitor DNA replication. Recent works have reported certain levels of cytotoxicity in mammalian cells, something that has never been evidenced in Trypanosomatids. To investigate the possible toxicity of these compounds in Trypanosomatids, we performed several kinetic assays using different concentrations of EdU and BrdU. For *T. cruzi*, we observed a reduction in proliferation and viability after 24h of EdU incorporation (50 and 100µM), but not after BrdU (50 and 100µM) incorporation. Furthermore, after 72h of compound withdrawal (recovery), the groups treated with the lowest doses of EdU and BrdU (10 µM) recovered, unlike those treated with the highest doses of EdU (50 and 100 µM). Regarding DNA content, we found no differences in the cell cycle phases at 1–24h of treatment. However, after 72h of the recovery period, we observed an accumulation of cells in the G1/S transition for the groups treated with the highest concentrations of EdU (50 and 100 µM), but not for those treated with BrdU. We also observed that after 72h of recovery, EdU remained incorporated into the DNA molecule in all groups treated. Currently, we are analyzing the presence of DNA damage through IFA using α-γH2A and TUNEL. Together, our data strongly suggest that EdU, but not BrdU, is toxic when used in high concentrations (>10µM). This finding is worrying, as the vast majority of works in the field use concentrations >10µM of EdU to monitor DNA replication in Trypanosomatids.

84 Cyclic AMP signaling impacts attachment of *Crithidia bombi*, a parasite of bumblebees, to surfaces in vitro

Sarah G Lockett (Villanova University); Blyssalyn V Bieber (Villanova University); Sonja K Glasser (University of Massachusetts Amherst); Neida O Portillo (University of Massachusetts Amherst); Faith A St. Clair (Villanova University); Madeline F Malfara (Villanova University); Michael Povelones (University of Pennsylvania); Lynn S Adler (University of Massachusetts Amherst); Megan L Povelones (Villanova University)

Crithidia bombi is a monoxenous Trypanosomatid parasite of bumble bees that imposes fitness costs on its insect host. The relationship between *C. bombi* and the North American bumblebee Bombus impatiens has long served as an important ecological model for host-pathogen interactions. We are using cell and molecular approaches to describe infection mechanisms and inform predictive models of pathogen spread in field conditions. To do this, we have genetically modified *C. bombi* to express fluorescent episomal transgenes. We have then used these cell lines to track infections in laboratory bumblebees. Like the closely related mosquito parasite Crithidia fasciculata and the human pathogen *Trypanosoma cruzi*, *C. bombi* uses its flagellum to attach to the lining of the hindgut within its insect host. These species will also attach to artificial substrates, allowing for quantitative, time-resolved studies of attachment efficiency. We have used an in vitro system to show that cyclic AMP (cAMP)-mediated signal transduction proteins are differentially regulated in swimming and attached C. fasciculata. Attachment of C. fasciculata in vitro can also be prevented by the cAMP phosphodiesterase inhibitor CpdA/NPD-001. Unusually for a Trypanosomatid, the genome of *C. bombi* predicts only one adenylate cyclase (AC) for enzymatic production of cAMP from ATP. Further, this AC has no predicted transmembrane or extracellular domain. Despite these differences, in vitro attachment of *C. bombi* is also sensitive to NPD-001 without impacting cell growth. These data show that the regulation of attachment by cAMP dynamics is broadly conserved in Trypanosomatids.

85 Structures of mitochondrial RNA editing helicase complexes

Shiheng Liu (University of California, Los Angeles); Takuma Suematsu (Boston University Medical Campus); Andres Vacas (Boston University Medical Campus); Inna Afasizheva (Boston University Medical Campus); Clinton Yu (University of California, Irvine); Lan Huang (University of California, Irvine); Xiaojing Zhao (ShanghaiTech University); Liye Zhang (ShanghaiTech University); Ruslan Afasizhev (Boston University Medical Campus); Z. Hong Zhou (University of California, Los Angeles)

In trypanosome mitochondria, uridine insertion/deletion RNA editing converts cryptic transcripts into functional messenger RNAs (mRNAs). RNA editing catalytic complex (RECC) executes a series of enzymatic reactions on gRNA/mRNA hybrid scaffolded by the RNA editing substrates binding complex (RESC). The editing process converts an imperfect gRNA hybrid with pre-edited mRNA into a ~50 bp nearly perfect duplex with edited mRNA. It follows that gRNA must be displaced to enable the binding of subsequent gRNAs during pan-editing and to allow translation of the fully edited transcript. The RNA Editing Helicase 2 Complex (KREH2C), consisting of the DEAH/RHA RNA helicase KREH2 and associated factors KH2F1 and KH2F2, has been implicated in the editing progression. However, the molecular mechanism of KREH2C action remains elusive due to the absence of high-resolution structures. Here, through cryo-electron microscopy and functional studies, we have discovered new factors and captured multiple states of KREH2C ranging from 0.4 to 0.7 MDa and determined their high-resolution structures. We will present KREH2C assembly dynamics and conformational changes underlying the gRNA translocation, a driving force of RNA editing complex remodeling.

86 A novel approach identifies transcriptome signatures across *Leishmania* spp. developmental stages and disease presentations

Ashton Trey Belew (University of Maryland, College Park); Najib El-Sayed (University of Maryland, College Park)

The advent of DNA and RNA-seq technologies has enabled considerable advances in the characterization of gene expression in *Leishmania* parasites. However, the genetic diversity among the 21 known *Leishmania* species has hindered in-depth comparative analyses. We have conceived a novel computational approach and applied it to the comparative analysis of the genome and transcriptome data from 8 *Leishmania* species. Using publicly available RNA-seq datasets, we then characterized gene expression globally across species and developmental stages. As expected, principal component analysis separated samples primarily along developmental stages. Differential expression (DE) analysis of amastigote versus promastigote stages across all species showed 29 upregulated and 630 downregulated genes. All DE genes had significantly greater rates of polymorphisms than constitutively expressed genes. Upregulated genes included those encoding amastins as well as proteins involved in apoptosis inhibition. Downregulated genes were consistently involved in gene regulation and motility factors. Finally, we compared transcriptome differences across *Leishmania* parasites with different clinical manifestations. Expression data from species that cause diffuse cutaneous Leishmaniasis (DCL) showed dramatic downregulation of many virulence-related proteins (e.g. tryparedoxin peroxidases, surface antigens), partially explaining the lower inflammation associated with DCL. Mucocutaneous Leishmaniasis (MCL)-causing species showed an upregulation of known immunogens (e.g. amastins, leishmanolysin). Gene expression patterns in visceral Leishmaniasis-causing species were characterized by increased amino acid catabolism alongside an overall increase in translation. Together, these results provide a reference for future work in comparative -omics approaches while providing insight into the conserved and divergent expression patterns within *Leishmania*.

87 Depletion of a single IP6K allele leads *Trypanosoma cruzi* to a quiescence-like state

Bryan Etindi Abuchery (Universidade de São Paulo (USP)); Thaise Lara Texeira (Universidade de São Paulo (USP)); Vitor Luiz da Silva (Universidade de São Paulo (USP)); Bruno Alves Santarossa (Instituto Butantã); Miguel Antonio do Nascimento Garcia (Universidade Federal de São Paulo (UNIFESP)); Maria Cristina M. Motta (Universidade Federal de Rio de Janeiro (UFRJ)); Samuel C. Texeira (Universidade Federal de Uberlândia (UFU)); Nilmar S. Moretti (Universidade Federal de São Paulo (UNIFESP)); Eloisa A. V. Ferro (Universidade Federal de Uberlândia (UFU)); Simone Guedes Calderano (Instituto Butantã); Miguel A. Chiurillo (University of Cincinnati (UC)); Noelia M. Lander (University of Cincinnati (UC)); Roberto Docampo (University of Georgia (UoG)); Marcelo Santos da Silva (Universidade de São Paulo (USP))

Inositol pyrophosphates (PP-IPs) – mainly IP7, and IP8 – are involved in a wide range of processes in eukaryotes. However, the mechanism of action of these metabolites is not yet fully understood. IP7 and IP8 are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids have an ortholog gene for IP6K, but do not have orthologs for PP-IP5K, making them excellent models for studying IP7. Here, using the CRISPR/Cas9 approach, we overexpressed IP6K and depleted single and double alleles of this kinase in *Trypanosoma cruzi* (the etiological agent of Chagas disease), generating IP6K+/+/+, IP6K-/+, and IP6K-/- lineages. IP6K inactivation causes several morphological effects, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, the IP6K-/- lineage was unable to proliferate, suggesting IP6K is essential to this organism. Curiously, IP6K-/+ lineage showed a slight cell cycle arrest at G0/G1 phase with no DNA damage. After developing an assay based on negative EdU (5-Ethynyl-2'-deoxyuridine) labeling, we found an increase in the number of quiescent cells in the IP6K-/+ lineage. This finding suggests that the presence of IP6K is important to keep *T. cruzi* committed to the cell cycle, i.e., the depletion of a single IP6K allele leads *T. cruzi* epimastigotes to a quiescence-like state. Together, our findings suggest that alterations in PP-IPs levels have harmful consequences for *T. cruzi*, which points to this kinase as a potential target for drug development, given that its identity relative to its human homologs is ~15%.

88 The Tandem Zinc-fingers of KH2F1 and Putative Hydratase KH2F2 in the Helicase Complex REH2C Differentially Impact the Editing of Distinct Mitochondrial Transcripts in *Trypanosoma brucei* Development

Sunil K. Sharma (Texas A&M University); Tyler Rodshagen (Seattle Children's Research Institute); Cody Goode (Texas A&M University); Joshua Meehan (Texas A&M University); Lars O'Hara (Texas A&M University); Desmond Givens (Texas A&M University); Suzanne M. McDermott (Seattle Children's Research Institute); Jorge Cruz-Reyes (Texas A&M University)

U-indel RNA editing in procyclic-form (PCF) and bloodstream-form (BSF) *Trypanosoma brucei* is developmentally regulated and we recently reported the complex REH2C is crucial in stage-specific editing repression (also reported by us in this meeting). REH2C includes RNA helicase KREH2 and directly bound KH2F1 and KH2F2 protein cofactors, but the roles of eight zinc finger motifs in KH2F1 and a hydratase domain in KH2F2 are unknown. We applied conditional knockdown, site-specific mutagenesis, and functional complementation to assess determinants for cell growth, editing function, and regulation. We substituted variable basic residues in each finger to decrease overall positive charge while preserving the basic fold. We also mutated the Mg2+ binding site at the putative catalytic center of KH2F2. So far, we found that several individual KH2F1 zinc fingers are differentially required for BSF growth and/or editing of specific transcripts, including those that are developmentally regulated. KH2F2 is differentially required during development. AlphaFold3 predicted a structure of REH2C complex, including the positions of KH2F1 zinc fingers, at least six dsRBDs, and putative regulatory motifs in KREH2. Further work will examine the possible impacts of KH2F1 loss and mutation on REH2C protein interactions using CXMS, helicase function, and BSF-specific editing repression. Importantly, the mutations do not affect the abundance of other editing components. We began similar studies in PCF. Together our data suggest the KH2F1 zinc fingers contribute to substrate specificity and direct RNA substrates to KREH2. Thus, the helicase complex REH2C is a key driver of developmental editing regulation in *T. brucei*.

Poster Session B – Abstracts

Thursday, September 12

7:00PM

35 (TT) High-accuracy genome assembly reveals hotspots for large gene families in *Trypanosoma cruzi* chromosomes

Lissa Cruz-Saavedra (McGill University); Luiza Berenguer Antunes (McGill University); Mira Loock (McGill University); Igor Cestari (McGill University)

Trypanosoma cruzi exhibits high genomic plasticity characterized by aneuploidies and repetitive sequences encoding virulence factors. The complexity of this parasite genome and the diversity of strains pose challenges for reconstructing the complete nuclear genome, resulting in limited high-quality reference genomes. Multigene families, such as mucins and trans-sialidases, play a critical role during infection; however, their genomic organization and expression throughout the parasite life stage are poorly understood. We generated a de novo assembly of *T. cruzi* Sylvio-X10 strain genome using long-read HiFi PacBio sequencing and chromatin conformation capture with nanopore sequencing (Pore-C). We assembled 35 complete chromosomes, 33 exhibiting telomeres, and resolved both haplotypes. We found 17 megabase (Mb) size chromosomes, 14 varying between 0.5-1 Mb, and 4 smaller than 0.5 Mb. Moreover, 34 chromosomes are diploid, and one Mb-size chromosome is tetraploid. There is evidence of segmental aneuploidy in various chromosomes, indicating the plasticity of this parasite genome. We identified 13,579 genes, of which 36% are large multigene families annotated at the subtype level. Notably, two Mb-size chromosomes have ~80% of their sequences represented by repeats, mainly large multigene families, suggesting a hotspot for their diversification. Retrotransposons and the Disperse Gene Family were enriched at subtelomeric regions, whereas other multigene families were compartmentalized across chromosomes. Gene expression analysis by RNAseq and tandem mass tag labelling mass spectrometry are in progress for each *T. cruzi* life stage. The data provide hints on *T. cruzi* genome organization and the complexity in the distribution and expansion of large gene families.

36 (TT) Cross-linking and mass spectrometry uncovers chromatin compartment proteins and subtelomeric VSG gene silencing

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Chromatin organization is essential in regulating gene expression in eukaryotes. In *Trypanosoma brucei*, chromosomes are organized into RNA polymerase II transcribed regions (core) and subtelomeric non-transcribed regions. This parasite has over 2,500 VSG genes and pseudogenes spread throughout subtelomeric regions used for VSG recombination during antigenic variation. We found that the enzyme phosphatidylinositol 5-phosphatase (PIP5Pase) and repressor-activated protein 1 (RAP1) interact and function in silencing VSGs from telomeric expression sites. However, the mechanisms by which hundreds of subtelomeric VSG genes are silenced remain unknown. Using in vivo chemical cross-linking, immunoprecipitation, and mass spectrometry, we mapped the interactions of PIP5Pase, RAP1 and their co-interacting proteins at peptide resolution within a radius of 11 Å. The data comprises over 10,000 crosslinked peptides. It revealed a large network of protein associations, including kinetochore, histones, cohesin complex, and chromatin-modifying enzymes, identifying their binding domains and revealing proteins that mediate multivalent interactions. Chromatin conformational capture (Hi-C) and ChIP-seq showed that RAP1 is enriched at the boundaries of chromosome compartments, often overlapping with sister chromatid cohesin (SCC1) and H3.V, indicating that interactions reflect protein composition at defined chromosome locations. Moreover, RAP1 spread over silent subtelomeric regions but not to transcribed chromosome core regions. PIP5Pase knockdown or mutation that inhibits PI(3,4,5)P3 dephosphorylation resulted in RAP1 removal from subtelomeric VSG genes and led to transcription of subtelomeric genes. The data indicates a network of protein interactions at the chromosome compartment boundaries, likely coordinating chromosome three-dimentional organization, and provides a mechanism for silencing subtelomeric VSG genes.

89 TblncRNA-23, a long non-coding RNA transcribed by RNA polymerase I, regulates developmental changes in *Trypanosoma brucei*

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The protozoan parasite *Trypanosoma brucei* undergoes a complex life cycle, moving between its mammalian host and the blood-feeding tsetse fly vector. The two major surface proteins expressed by procyclic forms in the insect midgut, EP and GPEET procyclin, are transcribed from a polycistronic transcription unit by RNA polymerase I. We identified a long non-coding RNA, termed TblncRNA-23, that is encoded between the two procyclin genes. TblncRNA-23 was localized to the nucleolus and also associated with polysomes. Overexpression of TblncRNA-23 identified EP and GPEET mRNAs as targets, as well as other mRNAs that changed abundance in the transition from early to late procyclic forms. TblncRNA-23 interacted with its substrates via base-pairing using different domains. Purification of TblncRNA-23-associated proteins by RaPID identified hundreds of proteins, including proteins translated from its target mRNAs, suggesting association with translating ribosomes. Nucleolar TblncRNA-23 bound multiple pre-rRNA processing factors, and cytoplasmic TblncRNA-23 associated with RNA binding proteins that regulate mRNA stability and translation. Early and late procyclic forms differ in their social motility (SoMo) capabilities, which is essential for migration away from the insect midgut to enable parasite transmission. Overexpression of TblncRNA-23 resulted in hypersocial motility, suggesting a regulatory role in SoMo and the parasite's potential to cycle between hosts.

90 Roles of *Trypanosoma brucei* POLIE and PPL2 in Telomere End Processing and Maintenance of Telomere Integrity

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Trypanosoma brucei, the protozoan parasite responsible for African trypanosomiasis, evades host immune surveillance by frequently changing its major surface antigen, Variant Surface Glycoprotein (VSG). Despite possessing a large reservoir of VSG genes, only one is transcribed at a time from subtelomeric expression sites by RNA polymerase I. Our research has demonstrated crucial roles of telomere proteins in regulating antigenic variation. These proteins maintain genome integrity, stabilize telomeres and subtelomeres, and suppress VSG switching. Telomeres, essential nucleoprotein complexes at chromosome ends, pose a challenge for DNA replication as conventional DNA polymerases cannot fully replicate linear DNA ends, leading to progressive telomere shortening. Telomerase, a specialized reverse transcriptase, addresses this issue by synthesizing the G-rich telomeric strand using a short template from its internal RNA component. However, telomere end processing mechanisms remain poorly understood. In *T. brucei*, telomeres end with a single-stranded 3'-overhang, crucial for forming a protective T-loops structure and acting as a substrate for telomerase. Maintaining this structure involves coordination between telomerase-mediated G-strand synthesis and DNA polymerase α/primase-mediated C-strand fill-in. Unlike mammals and yeasts, *T. brucei* lacks OB-fold-containing ssDNA binding telomere-specific proteins. We have found that POLIE, an A-type DNA polymerase, inhibits telomerase-mediated G-strand elongation and promotes C-strand fill-in. We established a *T. brucei* strain with a floxed POLIE allele to examine POLIE mutant phenotypes. Additionally, we identified PPL2 as a critical factor in coordinating G- and C-strand synthesis at *T. brucei* telomeres, alongside homologs of Apollo and EXO 1. Investigating telomere maintenance mechanisms in *T. brucei* will help better understand regulation of antigenic variation and evolution of telomere proteins.

91 Revisiting the impact of cruzipain inhibitor on *T. cruzi* egress

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Life-long infection by *Trypanosoma cruzi* underlies the development of human chronic Chagas disease causing severe cardiac, digestive, or neurological alterations. In the mammalian host, *T. cruzi* adopts an intracellular parasitic lifestyle. Following host cell invasion by trypomastigote, the parasite transforms to amastigote and replicates within the host cytoplasm. The end of the intracellular cycle is marked by the transformation of the amastigotes into motile trypomastigotes in a process called trypomastigogenesis, and by the lysis of the host cell resulting in the release of hundreds of infective parasites. These late steps play a crucial role in the pathogenesis of Chagas disease by promoting inflammation, dissemination, and transmission of the infection. Despite their importance, the cellular and molecular determinants underlying the late stages of the infection remain poorly understood. In this context, we aimed to dissect the trypomastigogenesis process and investigate the egress of *T. cruzi* parasites from the host cell. We employed ultra expansion microscopy to visualize and analyse the different steps of trypomastigogenesis within intracellular parasites and explore the transformation of amastigotes into trypomastigotes. We also developed an automated pipeline to efficiently assess parasite egress in *T. cruzi*-infected culture. We then used both methods to revisit the impact of the cruzipain inhibitor, Z-FA-FMK, on late infection events. Our assays provided valuable tools for studying egress, a critical yet poorly understood process in Chagas disease.

92 Inositol pyrophosphates are involved in DNA metabolism, pathogenicity, and virulence in Trypanosomatids

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In eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of cellular processes. However, the mechanism of action of these metabolites is not yet fully understood. Here, we deplete a single IP6K allele in *T. cruzi*, generating a PP-IPs-deficient *T. cruzi* lineage (IP6K-/+). Epimastigote forms of this *T. cruzi* lineage showed a slight cell cycle arrest at G1 phase with no DNA damage (increased dormant cells). Moreover, the *T. cruzi* IP6K-/+ lineage presented a reduced differentiation capacity during metacyclogenesis (impaired epi-meta transition) and a reduced invasion rate in human cardiomyocytes. Furthermore, the *T. cruzi* IP6K-/+ lineage showed an impaired ama-trypo transition within human cardiomyocytes. In *Leishmania braziliensis*, we generated different lineages KO for IP6K (IP6K-/+), telomerase (TERT-/-), and double-KO (IP6K-/-+TERT-/-) to investigate the role of PP-IPs in telomere homeostasis. The *L. braziliensis* double-KO lineage presented impaired proliferation and a slight cell cycle arrest at G2/M. Moreover, after the 10th passage, the *L. braziliensis* double-KO lineage presented DNA lesions, suggesting a telomere shortening. Curiously, these findings were not observed for the other *L. braziliensis* KO lineages. Transcriptomics assays are helping us identify possible pathways dependent on PP-IPs, both in *T. cruzi* and *L. braziliensis*. Together, our findings suggest that alterations in PP-IPs levels (caused by IP6K depletion) have harmful consequences for *T. cruzi* and *L. braziliensis*, especially when related to DNA metabolism (for *L. braziliensis*) and virulence/pathogenicity (for *T. cruzi*), pointing IP6K as a potential target for drug development, given that its identity relative to its human homologs is ~15%.

93 Discovery of an RNA binding complex from the antipodal nodes of *Trypanosoma brucei*

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The Kinetoplast Polyadenylation Complex (KPAC) regulates stability of mitochondrial mRNAs in *Trypanosoma brucei*. KPAC consists of five RNA-binding factors (KPAF1-5) and the poly(A) polymerase KPAP1. We have shown that pentatricopeptide repeat (PPR) factor KPAF4 and KPAF5 polypeptide lacking discernable motifs form a poly(A)-tail binding heterodimer. By assessing KPAF4/5 interactome, we predicted a stable interaction between KPAF5 and an uncharacterized protein which contains an N-terminal domain structurally homologous to MRP1 and MRP2, and an acidic disordered C-terminal region. The MRP1/2 RNA-binding heterotetramer has been extensively studied for more than 30 years but its function remains unclear. Here, we demonstrate that the newly discovered factor, named MRP3, forms a complex with MRP2, in which it displaces MRP1. We also provide evidence of MRP2/3 in vivo proximity to the KPAC. We show that MRP3 co-localizes with a fraction of MRP2 in the antipodal nodes while MRP1 and bulk of MRP2 are distributed in the matrix. Antipodal nodes represent membraneles-compartments flanking the kinetoplast and contain DNA-replication, primary RNA-processing, and other factors. We demonstrate that the C-terminal region determines MRP3 localization to the antipodal sites. Consistent with proximity to primary RNA-processing machinery, MRP3 binds to and stabilizes pre-edited and never-edited mRNAs but not ribosomal or guide RNAs. Furthermore, the MRP2/3 complex from the antipodal nodes is essential for viability of procyclic form of *T. brucei* while the matrix-localized MRP1/2 is not. The localization, RNA-binding properties, and loss-of-function impact on the transcriptome, suggests that MRP2/3 complex is the key mitochondrial mRNA stabilization factor in *T. brucei*.

94 LeishTag: Defining the subcellular localisations of the *Leishmania* specific proteome

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In their mammalian host *Leishmania* infect macrophages, yet the machinery that enables them to survive and replicate in this environment has not been defined in detail. Determining the subcellular localisation of this protein machinery is an important step, as this can be indicative of function. The subcellular localisation of nearly all the protein-coding genes in *Trypanosoma brucei* were determined in TrypTag; however, nearly a third (~2700/8267) of the proteins encoded in the *Leishmania* genome lack or have limited sequence identity (<30%) with those in *T. brucei*. This makes it difficult to infer the function of these proteins which appear to be recent evolutionary adaptations in *Leishmania*. LeishTag aims to provide subcellular localisations for these ~2,700 proteins by the fusion of mNeonGreen to their N or C termini. The resulting cell lines are imaged as promastigotes and axenic amastigotes, with the localisation manually annotated. As of June 2024, we have localised 1,230 proteins in both promastigotes and axenic amastigotes. Frequent protein localisations include the pellicular membrane and flagellar pocket region, suggesting these structures have undergone recent evolutionary adaptation, and may be important for mammalian pathogenicity. We have imaged 1,638 cell lines as both promastigotes and axenic amastigotes and in 275 instances the promastigote localisation differed to the amastigote, potentially indicating a role in macrophage infection. LeishTag is a community resource, data is made rapidly available at LeishTag.org, and is part of the LeishGEM project, which is generating complementary LOPIT - Localisation of Organelle Proteins by Isotope Tagging and deletion-mutant fitness data.

95 The Role of Haptoglobin-Related Protein in Innate Immunity to African Trypanosomes

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African Trypanosomes are parasites that cause nagana in animals. Humans and some primates are innately protected against trypanosome infections due to specific high-density lipoproteins called Trypanosome Lytic Factors (TLF). TLFs contain distinct proteins: haptoglobin-related protein (HPR) and apolipoprotein-L1 (APOL1). APOL1 is a cation-channel-forming protein that lyses trypanosomes through ion dysregulation and osmotic swelling. HPR is >90% homologous to haptoglobin (Hp), which binds to and promotes degradation of free hemoglobin (Hb). Trypanosomes are heme auxotrophs and endocytose Hp:Hb complexes using cell surface haptoglobin-hemoglobin receptors (HpHbR). HPR, secreted bound to TLF due to an uncleaved hydrophobic signal peptide, also binds Hb and exploits HpHbR, resulting in endocytosis of TLF and delivery of the Trojan horse, APOL1, to effect parasite lysis. HPR encodes an inactive serine protease domain. The catalytic triad of serine proteases is aspartic acid(D), histidine(H), and serine(S). Two residues in the HPR “inactive proteolytic pocket,” K144 and A297, are predicted to inhibit enzymatic activity. I hypothesize that HPR uses the inactive proteolytic pocket to bind APOL1, protecting it from degradation. Using site-directed-mutagenesis (SDM), I will engineer catHPR, a recombinant, soluble form of HPR (L20V & Y21D generates a cleavable signal peptide) with restored proteolytic function (K144H & A297S) using a mammalian expression vector. Purified catHPR enzymatic activity from transfected Chinese Hamster-Ovary (CHO) cells will be evaluated with recombinant APOL1 as a substrate, visualizing degradation by Western blot. This study will expand our understanding of human innate immunity against trypanosomes and address the role of HPR in TLF.

96 Determining the role of sodium accumulation in TLF-mediated lysis of African Trypanosomes

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African trypanosomes are unicellular, eukaryotic parasites that infect humans and cattle. 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). The mechanism of APOL1-mediated lysis remains controversial. We propose that after receptor-mediated endocytosis, APOL1 is inserted in the endosomal membrane due to the acidic pH, forming a closed ion channel. Once the endosome is recycled to the neutral environment of the plasma membrane, the channel opens and ions move down their electrochemical gradient. Sodium and calcium ions influx through APOL1, potassium ions efflux through leak channels, and chloride ions influx through chloride channels causing an osmotic imbalance, resulting in the trypanosome accumulating water, swelling, and bursting. Other models suggest APOL1 is trafficked to the mitochondria, opening a megapore and causing mitochondrial membrane depolarization. We hypothesize that the mitochondrial membrane depolarizes due to the sodium influx through APOL1 at the plasma membrane. To test this hypothesis, flow cytometry was used, with fluorescent indicators ING2-AM, a sodium-specific dye, and TMRE to measure mitochondrial membrane depolarization in response to TLF. Within 30 minutes of TLF treatment, sodium accumulation, and mitochondrial membrane depolarization were detected. When replacing extracellular sodium with tetramethylammonium (TMA+) a larger cation, less mitochondrial depolarization was observed. These results suggest that sodium influx (plasma membrane depolarization) plays a role in causing mitochondrial membrane depolarization. To further understand the role of ions in this lysis mechanism, we will block the potassium leak channels (TbK1) with RNAi, and measure the effects of potassium ions on mitochondrial membrane depolarization.

97 Investigating the Interaction of TbICP and TbCatL Recombinant Protein in a CHO-S Mammalian model

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Trypanosoma brucei, a eukaryotic parasite, can cause African sleeping sickness in humans and nagana in cattle. Human resistance to most subspecies is attributed to Trypanosome Lytic Factor, with the apolipoprotein APOL1 causing lysis in the parasite by forming channels in its membrane. *T. brucei*'s lysosomal cysteine protease, Cathepsin L (TbCatL), degrades APOL1, inhibiting lysis. The presence of Inhibitor of Cysteine Proteases (TbICP) in trypanosomes is thought to modulate TbCatL, but the in-vitro interaction between TbICP (cytosolic) and TbCatL (lysosomal) is not well-understood. This project aims to purify, quantify, and analyze both TbICP and TbCatL through in-vitro cotransfection in mammalian cells, as well as study their effect on APOL1. To attain our goal, we will transfect Chinese Hamster Ovarian suspension cells (CHO-S) with pcDNA plasmid containing HIS-epitope-tagged TbICP and TbCatL, followed by Nickel-column chromatography for protein purification. Enzymatic assays will be used to analyze protein activity in fractions, while Western Blot and Silver staining will determine the presence of the proteins of interest. The Bicinchoninic acid (BCA) assay will determine the expressed protein concentration in our model. We have purified and isolated TbICP and TbCatL from the media of CHO-S cell transfections. We plan to analyze the enzymatic activity of TbCatL in the presence of TbICP at various pHs. Using our in-vitro system, we aim to understand the interaction between purified recombinant TbCatL and TbICP through enzymatic analysis. Furthermore, we aim to derive how TbCatL and TbICP may interact and modulate APOL1 function, using our purified proteins.

98 Elucidating ICP Localization in *Trypanosoma brucei*

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Trypanosoma brucei are eukaryotic parasites that cause Human and Animal African Trypanosomiasis. Humans and some primates have an innate immunity complex, Trypanosome Lytic Factor (TLF), whose lytic component Apolipoprotein-L1 (APOL1), forms ion channels in the parasite's plasma membranes, causing ion imbalance resulting in osmotic lysis. Trypanosomes have an essential lysosomal protease, Cathepsin-L (CatL), which degrades APOL1. ICP (Inhibitor of Cysteine Peptidase) inhibits CatL, preventing APOL1 degradation, suggesting that APOL1 forms channels in lysosomal membranes. Previous research showed that ICP knockdown increases parasite resistance to APOL1/TLF-mediated lysis. This is based on the untested assumption that ICP and CatL interact in the lysosome. ICP is synthesized in the cytosol and CatL in the Endoplasmic Reticulum. It is unclear how these two soluble proteins interact when separated by a membrane. We hypothesize that ICP is present throughout the cell cytosol, and knockdown could free lysosomal escaped CatL to degrade APOL1/TLF elsewhere. To determine whether ICP is bound to CatL within or outside of the lysosome, we will pull-down the whole lysosome (Lyso-IP) using p67 specific antibodies, p67 is a lysosomal transmembrane protein, and probe for ICP. We generated a T.b.brucei cell-line expressing Cas9 on a tetracycline-inducible T7-promoter, 2T1PT7;Cas9. We introduced a stably transfected sgRNA (single-guide RNA) for ICP, then introduced a repair template to incorporate an HA-tag-ICP. Upon confirmation of properly introduced tags through immunoblot and immunofluorescence, we will complete the Lyso-IP. If ICP is in the non-lysosomal fraction, it would suggest that ICP is interacting with CatL released from the lysosome, which will allow us to better understand its role in regulating APOL1-mediated lysis.

99 DNA replication compartmentalisation dictates genome stability and instability in *Trypanosoma brucei*

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The genome of *Trypanosoma brucei* is structurally complex. Eleven megabase-sized chromosomes each comprise a transcribed core flanked by silent subtelomeres, housing thousands of Variant Surface Glycoprotein (VSG) genes. Additionally, silent VSGs are also found on hundreds of sub-megabase chromosomes that harbour 177 bp repeats of unknown function, and multiple VSG transcription sites localise to the telomeres of both chromosome types. DNA replication dynamics have been described in the megabase chromosome cores but not in the subtelomeres or sub-megabase chromosomes, and targeted early replication of the single active VSG transcription site is unexplained. Using Nanopore assembly, we mapped DNA replication across this compartmentalised genome. We show that subtelomeres display a paucity of replication initiation events relative to the core, correlating with increased instability in the silent VSG archive. In addition, early replication of the active VSG transcription site is shown to originate from the telomere, likely causing targeted VSG recombination. Lastly, we demonstrate that the 177 bp repeats act as widespread, conserved DNA replication origins, explaining mitotic stability of the abundant small chromosomes and early DNA replication of megabase chromosome centromeres. Compartmentalized DNA replication dynamics therefore explains how *T. brucei* balances stable genome transmission with localised instability driving immune evasion.

100 Trypanosomatid parasites as a model for termination of translation and stop codon read-through

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Translation is terminated when a stop codon is recognized by the protein release factor 1 (eRF1), which binds into the A-site of a translating ribosome. Next, it is accompanied by a GTPase called release factor 3 (eRF3). However, the principles of translation termination remain unknown in the newly described Trypanosomatid *Blastocrithidia* nonstop containing a non-canonical genetic code with all three stop codons reassigned for amino acids. Here, we aim to characterize translation termination in *Trypanosoma brucei* and use it as a model for B. nonstop. The two proteins eRF1 and eRF3 are essential in *T. brucei*, as their depletion by RNAi caused lethal phenotypes, and led to an increased stop codon read-through in the dual reporter assay. Additionally, we observed global defects in translation by polysome profiling after depletion of each eRF. We have identified multiple substitutions in the eRF proteins of B. nonstop in otherwise conserved residues, suggesting a role in decoding of the non-canonical genetic code. For instance, in eRF1 Ser67Ala is present in the stop codon binding pocket and contributes to stop codon read-through. Our heterologous system provides an optimal tool to test similar substitutions and other features identified in B. nonstop, and their contributions to translation termination efficiency and fidelity. We believe that this research is highly relevant to human genetic diseases associated with the appearance of premature termination codons.

101 Role of the RNA binding protein RBP42 in *Trypanosoma brucei* bloodstream form parasites

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In the absence of regulated transcription of individual genes, post-transcriptional processes play pivotal roles in *T. brucei* gene regulation. Various RNA binding proteins (RBPs) that interact with specific sets of mRNAs are the main drivers of this regulation. However, we are just beginning to understand how RNA binding proteins function in these parasites to accomplish the extensive and intricately networked gene expression patterns. *T. brucei* RBP42 is an essential RNA-binding protein that mainly localize in the cytoplasm and is tightly associated with polysomes. Previous studies have shown that in procyclic *T. brucei* RBP42 preferentially associates within the coding sequence of mRNAs involved in cellular energy metabolism. Our current study in bloodstream form parasites extends our knowledge of RBP42's role in the regulating cellular metabolism. Using individual-nucleotide resolution iCLIP studies we observed binding of RBP42 within the coding region of mRNA encoding metabolic enzymes, like procyclic parasites. Using a conditional knockdown cell line of BF *T. brucei*, we tested global changes in the transcriptomes and proteomes following RBP42 depletion. While no significant changes in RBP42-target mRNAs were recorded, the amount of RBP42-target mRNA-encoded proteins reduced significantly, indicating a role of RBP42 in translational regulation of target mRNAs. We also observed significant changes in metabolic intermediates of the cellular energy metabolic pathways following RBP42 depletion. Our data indicates a critical role of RBP42 in regulation of cellular energy metabolism of the parasite. Currently we are performing assays to find out the exact mode of translational regulation by RBP42. These data will be presented.

102 Can we visualize Apolipoprotein L1 at the plasma membrane of African Trypanosomes?

Kayla Leiss (Department of Biological Sciences, Hunter College-CUNY); Sara Fresard (Biology Program, The Graduate Center CUNY); Jayne Raper (Department of Biological Sciences, Hunter College-CUNY)

African trypanosomes are flagellated, extracellular parasites that infect humans and cattle. Trypanosome Lytic Factor (TLF), a subset of high-density lipoproteins (HDL), provides immunity to some species of trypanosomes in humans and some higher order primates. Two unique proteins on TLF include haptoglobin-related protein (HPR), a ligand for a parasite receptor, and Apolipoprotein-L1 (APOL1), a lytic protein. The mechanism of lysis is controversial. We hypothesize that APOL1 is trafficked through the endocytic pathway, forming an open pH-gated cation channel upon recycling to the plasma membrane, resulting in osmotic lysis. Two competing models of lysis hypothesize that APOL1 forms anion channels in the lysosome or a pore in the mitochondrial membrane. To determine the localization of APOL1 channel formation, a fluorescent tag can be used to track the protein in parasites via fluorescent microscopy. SNAP-tag (19 kDa) binds specifically and irreversibly to fluorescent benzylguanine derivatives. We generated a bacterial expression vector and confirmed that purified recombinant SNAP-tagged APOL1 can efficiently lyse trypanosomes. For the physiological characteristics of APOL1 embedded in an HDL, we are using a transient transgenic murine model for SNAP-tagged APOL1 expression. We generated a mammalian expression vector containing SNAP-tagged APOL1 and HPR, and with hydrodynamic gene delivery, expression occurs in the liver where HDL is assembled and secreted. We will treat trypanosomes with the purified HDL, and use confocal microscopy to localize SNAP-tagged APOL1, and co-stain with lysosomal, mitochondrial and plasma membrane markers. Ultimately, the results of this experiment will provide crucial data for understanding where APOL1 channel formation occurs.

103 TbICP, where are you? Intracellular localization of the Inhibitor of the Cathepsin L of *Trypanosoma brucei*

Bernardo Gonzalez (Hunter College); Nyhal Metidji (Hunter College); Kayla Leiss (Hunter College); Daniel Lopes (Hunter College); Jayne Raper (Hunter College)

Trypanosomiasis affects humans as well as domestic and wildlife animals worldwide. However, humans and some non-human primates can eliminate many trypanosome species once infection occurs due to trypanosome lytic factors, TLFs, a high-density lipoprotein comprising several macromolecules, including apolipoproteinL1,ApoL1. ApoL1 produces pH-dependent cation channels in trypanosome membranes, generating an ion and osmotic imbalance in the parasite and subsequent lysis of the parasite. We are focused on the intracellular mechanisms that regulate ApoL1 function in the parasite. It has been proposed that the lysosomal enzyme Cathepsin L (TbCatL) and its endogenous inhibitor, TbICP (*Trypanosoma brucei* Inhibitor of Cysteine Peptidase), modulate the trypanolytic activity of ApoL1 within the trypanosome lysosome. However, to date, there is no direct evidence of the interaction of TbCatL- TbICP in the lysosome, and there is no evidence indicating the presence of TbICP in this organelle. Our project seeks to elucidate where TbCatL-TbICP interaction could occur in the trypanosome. To answer these questions, we first wanted to determine the location of TbICP in the trypanosome tagging the endogenous TbICP, using a tagging inducible CRISPr-Cas9 system. Briefly, we transfected a 2T1 *Trypanosoma brucei* parental cell line with TbICP sgRNA plasmid to knock out the TbICP gene and induce double-strand break (DSB) and then introduced a repair template with the tag. Preliminary immunofluorescence localization analysis of TbICP suggests that the possible interaction between ICP and Cathepsin L is not in the lysosome, but more studies must be done.

104 tRNATyr Has an Unusually Short Half-Life in *Trypanosoma brucei*

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Following transcription, tRNAs undergo a series of processing and modification events to become functional adaptors in protein synthesis. Eukaryotes have also evolved intracellular transport systems whereby nucleus-encoded tRNAs may travel out and into the nucleus. In trypanosomes, nearly all tRNAs are also imported from the cytoplasm into the mitochondrion, which lacks tRNA genes. Differential subcellular localization of the cytoplasmic splicing machinery and a nuclear enzyme responsible for queuosine (Q) modification at the anticodon "wobble" position appear to be important quality control mechanisms for tRNATyr, the only intron-containing tRNA in *T. brucei*. Since tRNA-guanine transglycosylase (TGT), the enzyme responsible for Q formation, cannot act on an intron-containing tRNA, retrograde nuclear transport is an essential step in maturation. Unlike maturation/processing pathways, the general mechanisms of tRNA stabilization and degradation in *T. brucei* are poorly understood. Using a combination of cellular and molecular approaches, we show that tRNATyr has an unusually short half-life. tRNATyr, and in addition tRNAAsp, also show the presence of slow-migrating bands during electrophoresis; we term these conformers: alt-tRNATyr and alt-tRNAAsp, respectively. Although we do not know the chemical or structural nature of these conformers, alt-tRNATyr has a short half-life resembling that of tRNATyr; the same is not true for alt-tRNAAsp. We also show that RRP44, which is usually an exosome subunit in other organisms, is involved in tRNA degradation of the only intron-containing tRNA in *T. brucei* and is partly responsible for its unusually short half-life.

105 To ligate or not to ligate: A splicing-independent function for the essential TRL1 homolog in *Trypanosoma brucei*.

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Trypanosoma brucei (*T. brucei*), the parasite responsible for sleeping sickness, has a single intron-containing tRNA gene (tyrosyl tRNA, tRNA-Tyr), which undergoes splicing to form a mature tRNA; a reaction essential viability. This process involves a series of enzymatic steps, including exon ligation by the multi-domain tRNA Ligase (TRL1). *T. brucei* has an essential TRL1 homolog, which may participate in exon ligation. The presence of these homolog provides an opportunity to investigate possible additional functions of TRL1. Here, we demonstrate by using RNA interference (RNAi) that reduction in the expression of one homolog (we named KCPD) causes a growth defect. This growth defect can be rescued by expressing a recoded copy that is resistant to RNAi, confirming that KCPD is an essential gene. However, depletion of KCPD did not cause accumulation of splicing intermediates or reduction in mature tRNA-Tyr. Expressing an intron-less tRNA-Tyr failed to rescue the growth defect, suggesting an essential function unrelated to tRNA splicing. Independent disruption of either the kinase or phosphodiesterase domains, in the recoded rescue protein, recapitulates the RNAi growth defect indicating that both these domains are necessary for essentiality of this cytoplasmic protein. Global proteomics reveal a potential role for KCPD in flagellar biogenesis. In conclusion, we show that KCPD, a TRL1 homolog, is not involved in tRNA maturation and may have a non-traditional role associated with the flagella.

106 Exploring the role of KREPA6 and other OB-fold proteins in RNA editing

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Kinetoplastids including *Trypanosoma brucei* require mitochondrial RNA editing for survival. Catalytic complexes (CCs) perform the steps of editing. The CCs contain several catalysts and multiple essential non-catalytic proteins including KREPA1-6 which contain OB-folds as do other related editing proteins. The roles of the OB-fold containing CC proteins are not well understood but the smallest of these, KREPA6, which has a C-terminal domain with a predicted intrinsically disordered region (IDR) has been hypothesized to play a central role in CC structure and organization. Random-mutagenesis and complementation screening reveals single amino acid substitutions throughout the OB-fold which inhibit growth of bloodstream forms. The various amino acid substitutions have different effects on CC structural integrity suggesting that the KREPA6 OB-fold plays important roles in protein-protein interactions within the CCs. Some KREPA6 OB-fold substitutions severely disrupted CC structure, whereas others result in changes to the sedimentation profiles of KREPA6 and other OB-fold proteins when analyzed by glycerol gradient. Some mutations cause sedimentation of KREPA1 and KREPA3 at lower S than KREPA2, suggesting a role for the KREPA6 OB-fold in associating KREPA1 and KREPA3 within the complex. Additionally, deletion of the C-terminal IDR suggests a role for this part of KREPA6 in protein-protein interactions. RT-PCR and deep sequencing analysis of the various mutants reveals alterations of editing which sheds light on the roles of the different regions of KREPA6 during editing. These results are helping to advance the understanding of the roles of KREPA6 and other OB-fold proteins in RNA editing.

107 TcVPS23: ESCRT I complex acts in the EV biogenesis pathway and in immunopathology of *Trypanosoma cruzi* experimental infection

Nadjania Saraiva de Lira Silva (Universidade Federal de São Paulo); Ana Claudia Trocoli Torrecilhas (Universidade Federal de São Paulo); Sergio Schenkman (Universidade Federal de São Paulo)

Eukaryotic cell secretion involves the Endosomal Sorting Complex Required for Transport (ESCRT), which consists of four multi-subunit complexes, designated ESCRT-0, I, II, and III. This apparatus is similar in protozoa such as Plasmodium, *Trypanosoma*, *Leishmania*, Giardia, and Entamoeba, all causing harmful human diseases. One of these is Chagas disease, resulting from *Trypanosoma cruzi* infection, which in many cases produces myocardial fibrosis. Here we hypothesized that its development could depend on *T. cruzi* secreted components. We generated parasites by replacing one of the copies of the TcVPS23 gene, a crucial component of the ESCRT-I machinery, with the hygromycin resistance gene using CRISPR/Cas9 technology. In the infective trypomastigote form, which has strong secretory activity, this hemi-knockout displayed altered surface proteins, enhanced release of soluble proteins, and significantly reduced formation of extracellular vesicles. When inoculated in mice, the hemi-knockout showed reduced blood parasites and mast cell recruitment in the heart during the acute phase of infection with higher levels of IL6 and decreased IFN-γ levels in the systemic circulation. Moreover, the cardiac tissue of hemi-knockout-infected mice showed low IL1-β and IL6 levels. In the chronic phase, the TCVPS23 hemi-knockout had fewer mast cells, reduced IL6 level, and cardiac fibrosis compared to the parental parasite line. These findings show that TCVPS23 of ESCRT-I component by affecting the release of extracellular vesicles and soluble molecules, alters the immune response and possible myocardial fibrosis in the *T. cruzi*-infected host.

108 KRBP72 facilitates ATPase-dependent editing progression through a structural roadblock in ATPase subunit 6 mRNA

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Uridine insertion/deletion editing of mitochondrial mRNAs is a significant characteristic of kinetoplastid parasites, such as *Trypanosoma brucei*. This editing process is guided by trans-acting guide RNAs (gRNAs) and facilitated by RNA Editing Core Complexes (RECCs). The non-catalytic RNA Editing Substrate Binding Complex (RESC) plays a crucial role in coordinating the interactions among RECC, gRNA, and mRNA, as well as various auxiliary factors. Our study elucidates the fundamental role of KRBP72 an auxiliary factor in the editing of A6 RNA in T brucei, focusing on its distinct effects in the procyclic form (PCF) and bloodstream form (BSF) life stages. By generating RNAi cell line, we confirmed its depletion disrupts the progression of RNA editing rather than initiation, causing a significant pause at a stem-loop structure within the A6 mRNA. High-throughput sequencing identified a critical region between editing sites (ES) that relies on KRBP72 for efficient editing progression. Structural analysis suggested that this pausing is related to a stable stem-loop structure. Enhanced cross-linking and affinity purification (eCLAP) revealed KRBP72 binding sites on A6 pre-edited mRNA, particularly at the stem-loop region, implicating KRBP72 in maintaining proper RNA structure for editing. Our mutational analysis showed that while RNA binding by KRBP72 is dispensable, its ATPase activity is essential for its function. Furthermore, KRBP72 interacts with RESC components, particularly RESC12A, which promotes KRBP72's association with A6 mRNA. Our findings suggest that KRBP72, with its ATPase activity, facilitates the progression of editing through challenging RNA secondary structures, highlighting its crucial role in A6 mRNA editing machinery.

109 Functional significance of DRBD18 in mitochondrial RNA editing of *Trypanosoma brucei*

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Trypanosoma brucei, a protozoan parasite causing African trypanosomiasis, exhibits a distinctive RNA editing mechanism crucial for its survival and pathogenesis. This process involves the precise insertion and deletion of uridine (U in/del) residues, generating mature mitochondrial mRNA transcripts from cryptic precursors. Editing is directed by trans-acting gRNAs and catalyzed by RNA Editing Core Complexes (RECCs). The non-catalytic RNA Editing Substrate Binding Complex (RESC) coordinates interactions between RECC, gRNA and mRNA. *T. brucei* mitochondria encodes 18 protein-coding genes, out of which 12 need post-transcriptional modification by U in/del. While the holoenzyme orchestrating this process is well characterized, recent investigations have highlighted the role of accessory RNA binding proteins (RBPs) in modulating RNA editing in *T. brucei*, often in a transcript-specific manner. DRBD18 is a multifunctional RBP that reportedly impacts stability, processing, export and translation of nuclear-encoded mRNAs. However, mass spectrometry studies report DRBD18-RESC interactions, prompting us to investigate its role in mitochondrial U in/del RNA editing. In this study, we demonstrate the specific and RNA-dependent interaction of DRBD18 with the RESC through co-immunoprecipitation. RNA interference-mediated depletion of DRBD18 in procyclic form *T. brucei* results in a significant reduction in edited A6 and COIII mitochondrial transcripts; conversely, its overexpression leads to a notable increase in the same edited mRNAs. RNA immunoprecipitation/qRT-PCR analysis supports a direct role for DRBD18 in A6 and COIII mRNA editing. Finally, mutagenesis analysis suggests that hypomethylated, but not arginine methylated, DRBD18 is functional for promotion of these editing events. Collectively, these findings demonstrate that the predominantly nuclear and cytoplasmic DRBD18 directly facilitates the editing of A6 and COIII transcripts in procyclic *T. brucei*, constituting the first report of a mitochondrial function for DRBD18.

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

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Mitochondrial transcripts in *Trypanosoma brucei* require extensive uridine insertion/deletion RNA editing to generate translatable open reading frames. The RNA editing substrate binding complex (RESC) serves as the scaffold that coordinates the protein-protein and protein-RNA interactions during editing. RESC broadly contains two modules termed the guide RNA binding complex (GRBC) and the RNA editing mediator complex (REMC), as well as organizer proteins. How the protein and RNA components of RESC dynamically interact to facilitate editing is not well understood. Here, we examine the roles of organizer proteins, RESC8 and RESC14, in facilitating RESC dynamics. High-throughput sequencing of editing intermediates reveals an overlapping RESC8 and RESC14 function during editing progression across multiple transcripts. Blue native PAGE analysis demonstrates that RESC14 is essential for incorporation of RESC8 into a large RNA-containing complex, while RESC8 is important in recruiting a smaller ribonucleoprotein complex (RNP) to this large complex. Proximity labeling shows that RESC14 is important for stable RESC protein-protein interactions, as well as RESC-RECC associations. Together, our data support a model in which RESC14 is necessary for assembly of editing competent RESC through recruitment of an RNP containing RESC8, GRBC and gRNA to REMC and mRNA.

111 Analysis of introns in kinetoplastids: a novel intron-containing gene and the first known eukaryote with only trans-splicing

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Previously, cis-splicing in Trypanosomatids has been reported only in two genes, namely poly(A) polymerase and RNA helicase. Conversely, trans-splicing, which involves the attachment of a spliced leader sequence, is observed in nearly every protein-coding transcript. With a large dataset including all described Trypanosomatid genera as well as some of their relatives from the class Kinetoplastea, we revealed a new conserved intron-containing gene encoding an RNA-binding protein present in all analyzed taxa. Importantly, all three intron-containing genes code for RNA-interacting proteins, which may fine-tune expression of multiple genes, thus challenging the view that cis-splicing in Trypanosomatids is just an evolutionary relic. Moreover, we show that Perkinsela sp., a kinetoplastid endosymbiont of an amoeba, represents the first eukaryote completely lacking spliceosomal introns, but still preserving trans-splicing. Our analyses reveal evidence of reverse transcriptase-mediated intron loss in Kinetoplastea, extended conservation of 5’ splice sites, and presence of non-coding RNAs within a subset of retained Trypanosomatid introns. In addition, we address the evolution of U1 small nuclear RNA, one of the key components of the spliceosome.

112 Understanding the consequences of a possible crosstalk between inositol pyrophosphates (PP-IPs) and telomeric dynamics in *Leishmania braziliensis*

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Leishmaniases are a group of diseases caused by parasites of the *Leishmania* genus. Currently, the drugs available for the treatment of these diseases present side effects and contribute to the selection of persistent parasites, making the search for new treatments fundamental. In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of cellular processes, including telomere dynamics. However, the mechanism of action of these metabolites in this pathway still needs to be elucidated. Here, using the CRISPR/Cas9 system, we generated the following knockout (KO) lineages in *L. braziliensis*: IP6K (IP6K-/+), telomerase (TERT-/-), and a double-KO (IP6K-/- + TERT-/-). These lineages were confirmed by PCR and RT-qPCR. The double-KO lineage presented an impairment in cell proliferation and a slight cell cycle arrest at cytokinesis. Moreover, after the 5th passage, the double-KO lineage presented DNA lesions, suggesting a possible telomere shortening. Curiously, these findings were not observed for the other KO lineages. Together, our findings point to a clear crosstalk between PP-IPs and telomerase in *Leishmania*. Currently, we are trying to figure out if the DNA lesions observed are in telomeres using FISH-IFA, Southern blot, and Flow-FISH. Also, transcriptomics and infection assays using human macrophages are being performed to elucidate the pathways impaired by the depletion of IP6K and TERT, and to verify the virulence of the double-KO lineage. These data may pave the way for the development of specific therapies against Leishmaniases since kinases are excellent targets for drug development, and telomeres are the molecular clock of eukaryotic cells.

113 Molecular Insights into Immune and Tissue Repair Mechanisms Associated with Congenital Chagas

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Chagas disease causes 12000 deaths annually, with 30,000 new cases per year. It is estimated that 23% of these new cases result from congenital transmission. Approximately 5% of Chagas-positive pregnant women transmit the disease to their infants, but the specific factors influencing transmission risk remain unknown. To better understand the molecular mechanisms driving transmission, we recruited mothers in Santa Cruz Bolivia who transmitted the disease to their infants (transmitters), and Chagasic mothers who did not transmit the disease (non-transmitters). We performed RNA sequencing on both peripheral blood and placental tissue to identify molecular signatures associated with a higher transmission risk. Peripheral blood and placental tissue were analyzed in parallel to define local and systemic signatures of transmission. When comparing the placental tissue of transmitting and non-transmitting mothers, we found 298 differentially expressed genes (DEGs) that, by gene set enrichment analysis (GSEA) indicated an elevated inflammatory response in the placentas of transmitting mothers. Even though no significant DEGs were detected in peripheral blood, a GSEA highlighted changes in immune response and tissue remodeling proteins, potentially associated with placental tissue damage. Our findings suggest that transmitting mothers exhibit unique gene expression patterns indicative of tissue damage, remodeling, and an increased inflammatory response to the parasite. Moreover, our data suggest that peripheral blood could be valuable for uncovering biomarkers that predict congenital Chagas transmission risk. To determine if parasite genetic variation or complexity of infection influences host responses, we have designed a panel of single-copy genes to evaluate genomic parasite variability. We plan to use this panel to identify parasite genomic profiles associated with various levels of transmission, including placenta colonization, umbilical cord colonization, and vertical transmission. Understanding the host and parasite fa

114 Profiling the human IgG response to *T. brucei* spp. infections using a Pan-VSG phage display library

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Human African Trypanosomiasis (HAT) is a severe vector-borne disease endemic to Sub-Saharan Africa which is caused by two subspecies of protozoan parasites, *Trypanosoma brucei* gambiense and *Trypanosoma brucei* rhodesiense. All subspecies of *Trypanosoma brucei* are known to rely on the antigenic variation of their dense coat of variant surface glycoproteins (VSG) to evade host antibody recognition and clearance. Antigenic variation has been studied extensively using strains of animal infectious African trypanosomes as a model system and have shown that the parasite's ability to avoid clearance by antibody is likely critical to the maintenance of chronic infections. Studies of the anti-VSG antibody response in vivo have been limited by low-throughput approaches that allow for the analysis of response against only one or a few VSG proteins at a time. Moreover, it is unknown whether findings are generalizable between animal models and human infections. Here, we use phage immunoprecipitation sequencing (PhIP-seq), a high throughput method for screening antibody specificities against antigen peptides which have been cloned and expressed on the capsids of T7 bacteriophage. The Pan-VSG phage library consists of over 50,000 overlapping peptides tiled across nonredundant sequences collected from the database of all *T. brucei* VSGs sequenced to date. We measured IgG reactivity with PhIP-seq to characterize the response against VSG peptides in HAT patient serum by determining whether there is commonality between responses in different patients, which part of the VSG protein is most likely to be targeted by IgG, and quantifying the breadth of specificities targeted. Applying the PhIP-seq method to serum collected from T. b. gambiense and T. b. rhodesiense infected HAT patients has revealed major differences in the host responses between the subspecies.

115 *Leishmania* amazonensis aquaglyceroporin 1 is a glycosomal protein involved in antimonial resistance

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Leishmania encounters critical conditions within macrophages, notably a scarcity of essential nutrients such as iron, which triggers the differential expression of various genes. In silico analyses of these genes, focusing on glycosomal targeting sequences, revealed aquaglyceroporin 1 (aqp1) as a conserved gene modulated by iron availability, containing predicted transmembrane domains and a glycosomal addressing sequence in *L. amazonensis*. *Leishmania* glycosomes are unique Trypanosomatid organelles that compartmentalize glycolysis, β-oxidation of fatty acids, and several essential enzymes, including the iron-dependent superoxide dismutase B (Fe-SODB). Previously, AQP1 was characterizes as a porin involved in the transport of trivalent antimony (Sb III), water, glycerol, urea, dihydroxyacetone, methylglyoxal, and polyols. Additionally, AQP1 has been identified in distinct subcellular compartments in *L. major* and L. donovani. Given the central role of glycosomes in *Leishmania* and the diverse subcellular localization of AQP1 across species, our objective was to investigate AQP1´s role in *L. amazonensis*. Using immunofluorescence, we confirmed AQP1´s glycosomal localization, and employing the CRISPR/Cas9 strategy generated full knockout and add-back strains, which were validated by PCR analysis. The knockout of aqp1 impaired promastigote growth in vitro, particularly under heme-depleted conditions, induced parasite resistance to Sb III, and increased superoxide dismutase activity. Furthermore, AQP1 deficiency significantly reduced both in vitro and in vivo virulence. Further characterization of these mutant strains will elucidate the relationship between AQP1 function iron metabolism, and antimony resistance in *Leishmania*. This study will provide critical insights into potential therapeutic targets and underscore the importance of species identification in the treatment of Leishmaniases.

116 Amino Acid Permease 3 senses arginine availability and responds to temperature and pH changes in *Leishmania major*

Ricardo Andrade Zampieri (University of São Paulo); Romario Lopes Boy (University of São Paulo); Juliana Ide Aoki (State University of Campinas); Maria Fernanda Laranjeira Silva (University of São Paulo); Karl Erik Muller (Oslo University Hospital); Audun Helge Nerland (University of Bergen); Lucile Maria Floeter Winter (University of São Paulo)

Arginine is a crucial amino acid for macrophage defense mechanisms against pathogens and is also essential for *Leishmania* replication. In *Leishmania*, arginine uptake is primarily mediated by amino acid permease 3 (AAP3), a transporter well characterized in *L. amazonensis* and L. donovani. In these species, aap3 can sense the availability of arginine and changes in temperature and pH. However, it has not been well characterized in other species. The aap-3 locus appears structurally conserved among *Leishmania*, presenting two copies in tandem of the CDS per chromosome. In *L. major*, this locus is situated on the tetrasomic chromosome 31. Based on sequences obtained by PacBio sequencing and transcriptome data, we generated *L. major* knockout mutants for aap3 using CRISPR-Cas9 strategy. This study aimed to evaluate the impact of the absence of aap3 copies on arginine uptake and assess the infectivity of these mutants. Interestingly, the null knockouts (aap3-1-/-/-/- and aap3-2-/-/-/-) was viable in promastigote forms only when supplemented with putrescine, a product of arginine metabolism and an essential intermediate in the polyamine pathway. The single copy knockouts (aap3-1-/-/-/- or aap3-2-/-/-/-) showed differential gene expression modulation under arginine starvation and supplementation, as well as pH and temperature changes. These results indicate that the sensing mechanism is also observed in *L. major* and is dependent of the locus structure. A deeper understanding of the role of the aap3 gene in *Leishmania* physiology can improve our knowledge of its host interactions and aid in developing diagnostic and pharmacological strategies to combat Leishmaniases.

117 Translation of the non-canonical genetic code of *Blastocrithidia* nonstop

Julie Kovářová (Institute of Parasitology, Biology Centre, Czech Academy of Science); Nathalia Ballesteros Chitiva (Institute of Parasitology, Biology Centre, Czech Academy of Science); Zdeněk Paris (Institute of Parasitology, Biology Centre, Czech Academy of Science)

Blastocrithidia nonstop, a unicellular flagellate related to parasitic trypanosomes and *Leishmania*, has a unique genetic code in which all three stop codons are reassigned to code for amino acids. We have recently elucidated the basic mechanisms underlying this divergence (1). We show the presence of cognate tRNAs for UAG and UAA, while UGA is decoded by a near cognate tRNA-Trp (CCA) with a shortened 4-base pair (4bp) anticodon stem. Building on these findings, we identified editing of cytidine to uridine at position 19 of the tRNA-Trp, which inhibits the UGA read-through as shown by a heterologous dual luciferase reporter assay in *Trypanosoma brucei*. This affects only a subset of the 4-bp tRNA-Trp population, and therefore we propose this newly identified editing event as a regulatory step for stop codon translation. Bioinformatic genome analysis revealed that the UAA is the only true stop codon that must be arranged in multiple copies to allow proper termination. We have been applying advanced techniques such as ribosome profiling as an exploratory tool to elucidate not only the dynamics of reassigned stop codon readthrough but also detailed insights into the termination process in B. nonstop. Our research positions this enigmatic parasite as a novel and important model for uncovering unique translation mechanisms. Reference: (1) Kachale, A., Pavlíková, Z., Nenarokova, A. et al. Short tRNA anticodon stem and mutant eRF1 allow stop codon reassignment. Nature 613, 751–758 (2023)

Poster Session C – Abstracts

Friday, September 13

7:00PM

45 (TT) Genome analysis of *T. cruzi* field isolates offers the opportunity to study the effect of infection context on parasite genetic diversity

Jill Hakim (Johns Hopkins); Sneider Gutiérrez (Johns Hopkins); Edith Malaga (Universidad Cayetano Herida); Louisa Messenger (University of Arizona); Monica Mugnier (Johns Hopkins)

Trypanosoma cruzi is the causative agent of Chagas disease which kills 10,000 people annually. There has been little investigation into the parasite's highly adaptive genome, especially in strains infecting humans. Characterizing the genomes of clinical strains is critical to defining the role that the human host plays in the parasite's evolutionary adaptation. Here, we describe short read whole genome sequencing of 15 clinically isolated *T. cruzi* samples from different infection contexts: mothers at time of delivery, patients with Chagasic cardiomyopathy, and patients co-infected with HIV. We have produced gene level assemblies for each sample and categorize parasites into canonical discrete typing units (DTUs). Additionally, we uncovered clusters of genetically similar parasites within DTU groups, indicating underlying genetic population structures not captured by DTUs that may be explained by other, perhaps epidemiological or geographic, variables. Of particular interest are diverse and highly antigenic multi-copy gene families, which are understudied yet represent important diagnostic targets. We find these families are expanded and more diverse in clinically isolated specimens compared to lab adapted strains. Moreover, we find a repertoire of genes for each multi-copy gene family member found across all clinical isolates, but are found in none of the lab adapted strains, regardless of DTU. Finally, to identify genes that may be under purifying selection during human infection, we calculated pN/pS within each sample and across samples. Genes with high pN/pS across all samples include members of ABC transporter complex, intraflagellar transport protein 88, and other important genes. This represents the first comparison of whole genomes from such a wide array of clinical contexts. These results demonstrate the feasibility of large-scale *T. cruzi* whole genome studies, allowing further investigation into genetic features driving clinical manifestation of disease.

46 (TT) Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models

Sara Fresard (CUNY Hunter College, The Graduate Center at City University of New York); Kayla Leiss (CUNY Hunter College); Russell Thomson (CUNY Hunter College); Jayne Raper (CUNY Hunter College, The Graduate Center at City University of New York)

African Trypanosomes cause a major agricultural and economic burden due to their negative impact on cattle. Some primates are protected against 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)), for parasite receptor-mediated endocytosis. How these TLFs are assembled is unknown. To elucidate TLF assembly, we analyzed human serum, hepatocyte tissue cell culture, and generated targeted germline transgenic murine models. We used immunoaffinity and size exclusion chromatography to isolate all APOL1 complexes from serum. TLF 1 and TLF2 were isolated as well as a small TLF ~150 kDa complex, TLF3. We hypothesize that TLF3 is a nascent HDL synthesized in hepatocytes based on its size, density, lytic ability, and gene expression. In our transgenic TLF mice, the most robust protection from trypanosome infection is observed when APOL1 and HPR are expressed from ubiquitin promoters, supporting the idea that TLF is made and secreted by cells under the same conditions, perhaps the same cell. To test this, we evaluated the secreted proteins from a human hepatocyte cell line (HepG2). We detected robust co-assembly of APOL1 and APOA-I (HDL scaffold protein) by size fraction and immunoaffinity purification. We detect low levels of Hpr and hypothesize that APOL1, and sometimes HPR, are loaded onto HDL in/on the hepatocyte with sufficient lipids to generate a nascent HDL. The TLF3 complex is released into the blood and matures into TLF1 by accumulating lipids and more HPR. By understanding TLF biogenesis, we can use the appropriate promoters in transgenic models to generate cattle completely resistant to trypanosomiasis.

47 (TT) Host response to cutaneous Human African Trypanosomiasis

Rhiannon Heslop (University of Glasgow); Nono-Raymond Kuispond Swar (National Institute for Biomedical Research, Kinshasa); Anneli Cooper (University of Glasgow); Paul Capewell (University of Glasgow); Dieudonne Mumba Ngoyi (National Institute for Biomedical Research, Kinshasa); Annette MacLeod (University of Glasgow)

Dermal trypanosomes have been identified in both Gambiense Human African trypanosomiasis (gHAT) patients, who have bloodstream parasites, and asymptomatic seropositive individuals, who lack detectable bloodstream parasites. However the prevalence of cutaneous trypanosomiasis and how these parasites persist in the skin remains unknown.

Here we have serologically screened 123 individuals from gHAT foci in the DRC. Blood samples and skin biopsies were then examined for evidence of trypanosomes by molecular and immunohistological methods. RNAseq analysis was conducted on 30 skin biopsies from seropositive and seronegative individuals to investigate the host dermal response to trypanosome infection.

We found 64% of seropositive individuals had trypanosomes in their skin. Our cohort included 19 individuals with a previous gHAT diagnosis, of whom 14 had trypanosomes in their skin up to 13 years after clinically successful treatment. Host transcriptomic analysis of skin biopsies revealed that individuals clustered by presence of dermal trypanosomes. Expression patterns compared between uninfected (serology and histology negative) and infected (serology and histology positive) individuals detected 1113 differentially expressed genes and 170 enriched pathways which were predominantly involved in infection, inflammation, cell proliferation and barrier integrity.

Our results demonstrate that cutaneous trypanosomiasis is widely prevalent in gHAT foci and that ‘successful’ treatment does not guarantee the clearance of dermal trypanosomes. This highlights that skin analysis could be a valuable addition to clinical diagnosis and treatment monitoring of gHAT. This pilot study shows a clear local host response to dermal trypanosomes and identifies candidates for biomarkers that could be used in future gHAT diagnosis.

55 (TT) RNA viruses in *Blastocrithidia*: does a unique genetic code protect from viral infection?

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Trypanosomatids (Euglenozoa) are a diverse group of unicellular flagellates predominately infecting insects (monoxenous species) or circulating between insects and vertebrates or plants (dixenous species). Monoxenous Trypanosomatids harbor a wide range of RNA viruses belonging to the families Narnaviridae, Totiviridae, Qinviridae, Leishbuviridae, and a putative group of tombus-like viruses. In this work, we focus on the subfamily Blastocrithidiinae, a previously unexplored divergent group of monoxenous Trypanosomatids comprising two related genera: Obscuromonas and *Blastocrithidia*. Members of the genus *Blastocrithidia* employ a unique genetic code, in which all three stop codons are repurposed to encode amino acids, with TAA also used to terminate translation. Obscuromonas isolates studied here bear viruses of three families: Narnaviridae, Qinviridae, and Mitoviridae. The latter viral group is documented in Trypanosomatid flagellates for the first time. Importantly, no RNA viruses were detected in *Blastocrithidia* spp., arguing that recoded genetic code indeed provided a protection against viral infections. Nevertheless, we identified an endogenous viral element in the genome of *B. triatomae* indicating its past encounter(s) with tombus-like viruses.

56 (TT) REH2C Complex is the First Identified Regulatory Factor in Lifecycle Stage-specific RNA Editing Repression During *Trypanosoma brucei* Development

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U-indel RNA editing in procyclic-form (PCF) and bloodstream-form (BSF) *Trypanosoma brucei* is developmentally regulated; however, the mechanisms and factors driving this regulation have remained a critical open question since RNA editing was discovered. Editing directed by anti-sense gRNAs creates canonical protein-encoding mRNAs. Intriguingly, canonical editing occurs amid massive alternative non-canonical editing of unclear biological significance. The complex REH2C includes helicase KREH2 and its binding partners KH2F1 and KH2F2. We report here that REH2C is crucial for stage-specific editing control. Specifically, KREH2 promotes PCF- and BSF-specific editing repression at major early checkpoints in mRNAs encoding ND7 and cytochromes COX3 and CYb. KREH2-mediated repression involved opposite modulation of canonical and alternative "terminator" gRNA utilization, which derails canonical editing. In vitro differentiation recreated stage-specific repression. ND7 studies showed terminator-programmed editing installs a proposed repressive structure determined using DMS-MaPseq. KREH2-RNAi knockdown reverted canonical/terminator gRNA utilization and thus the repression phenotype. Transcripts that bypassed early termination were still repressed further upstream by a similar mechanism, suggesting global modulation of gRNA utilization. Terminators are novel "moonlighting" gRNAs also associated with canonical editing in their cognate mRNAs, reveling that the gRNA transcriptome is multifunctional. Notably, changes in the level of KH2F1 and KH2F2, which directly bind KREH2, can dramatically affect stage-specific editing repression. KREH2 is the first identified repressor in developmental editing control. This and our prior work support a model whereby KREH2 activates or represses editing in a stage and substrate-specific manner. KREH2's novel dual role tunes mitochondrial gene expression in either direction during development.

57 (TT) Deep mutational resistance profiling for anti-Trypanosomal proteasome inhibitors

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Although anti-infective drug resistance presents a major threat, characterisation of potential resistance-associated mutations often remains incomplete. Now that several new anti-Trypanosomal drugs, with known targets, are in clinical development, we aim to improve our understanding in this area. We developed oligo targeting for precision editing in otherwise wild-type Trypanosomatids (PMID:35524555) and have now scaled the approach for saturation mutagenesis of residues comprising a drug-binding pocket. The *Trypanosoma brucei* proteasome is currently a promising anti-Trypanosomal target (PMID:27501246, PMID:30962368), and twenty residues within 5Å of bound drug in cryo-EM structures were targeted for saturation mutagenesis, stepwise drug-selection, and amplicon-sequencing. Among 1,280 mutants in the pooled library, codon variant scoring revealed resistance 'hotspots', which aligned well with 'functional' mutational space, as determined by fitness profiling; edits of residues directly involved in catalysis failed to yield survivors, for example. Nevertheless, >100 distinct resistance-conferring base-edits and >45 distinct amino acid edits were recovered. This contrasts with only a small number of single nucleotide polymorphisms recovery following drug-selection without editing, providing insights into limits imposed within 'accessible' mutational space. The digital data yielded virtual dose-response curves, which were predictive of EC50 values derived in vitro using a bespoke panel of edited mutants (R2 = 0.98); resistance increased up to 100-fold relative to the 4 nM EC50 observed for wild-type cells. Iterative computational modelling, informed by the quantitative experimental data, revealed how specific steric constraints, charge differences and backbone interactions contributed to varying degrees of resistance. The methods and findings we describe have the potential to facilitate modelling of drug-target interactions, assessment of drug-resistance potential, and design of more efficacious and durable drugs.

58 (TT) Forging the One Ring of *Trypanosoma brucei*: Structural characterization of BILBO1 assembly

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The Flagellar Pocket (FP) of *Trypanosoma brucei* is essential for the parasite's survival and pathogenicity. It functions as the sole site for all endo- and exocytosis activities and is crucial in the clearance of host antibodies. The FP's biogenesis and maintenance rely heavily on the flagellar pocket collar (FPC), a ring-like structure located at the neck of the FP. The assembly of the FPC requires BILBO1, a multi-domain cytoskeletal protein. Previous studies show that RNAi depletion of BILBO1 causes cellular abnormalities, including the lack of new FP and FPC, leading to cell death. However, the mechanisms by which BILBO1 forms the FPC scaffold and regulates FPC assembly remain unclear. To explore this, we conducted structural studies on full-length and truncated BILBO1. We determined a 1.9-Å resolution crystal structure of the BILBO1 filament junction revealing that two leucine zipper motifs are arranged in an antiparallel fashion to connect neighboring BILBO1 coiled-coil dimers. This tight junction allows BILBO1 dimers to form long filamentous polymers. Electron microscopy of full-length BILBO1 showed that numerous filaments could laterally associate to form an extended belt-like structure, with globular domains aligned in registers to create distinct condensed stripes. Furthermore, calcium binding significantly modulates the rigidity and compactness of these stripes. Intriguingly, treating samples with a BILBO1-specific nanobody resulted in detaching filaments with loosely packed circular structures. Overall, these findings provide a mechanistic explanation for BILBO1 filament formation and bundling, offering a potential strategy for disrupting BILBO1 function in FPC assembly by targeting critical interfaces within the junction/bundles.

118 Endocytosis and Recycling of the Surface Protein Coat in *Trypanosoma brucei*

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Trypanosoma brucei evades host immune destruction by rapidly recycling surface coat molecules. The varaint surface glycoprotein (VSG) coat operates at the highest physically possible protein density. We refined a process to create artificial membranes with a high protein density, resembling diffusion speeds observed in in-vivo experiments of plasma-membrane anchored VSG. To match the highly dynamic surface, the internal cisternal complex also exhibits high surface dynamics, observable in single-molecule videos in 3D. The whole surface coat on the parasite is recycled approximately every 12 minutes (Engstler et al. 2004), facilitated by a continuous endomembrane system that enables swift sorting of molecules and are stabilized by actin-myosin skeleton (Link et al. 2023; Link et al. 2024). Tomographic data reveal an interconnected endosomal system for the sorting of surface molecules. Given that the rapid surface turnover must be paralleled by equally swift recycling or degradation, we have turned our focus to the endosomal retromer complex (ERC) in *T. brucei*. The ERC is primarily responsible for recycling membrane proteins from the endosomes to cell surface. We used immunoflurescence and electron microscopy to determine the localization of the four ERC subunits, which are confined to the endosomal compartment with the trimeric core retaining the characteristic arch structure. TbVps5, a sorting nexin, colocalizes with late endosomes, suggesting its involvement in sorting within the endolysosomal pathway. Our findings may significantly advance the general understanding of the endosomal recycling apparatus and elucidate the mechanisms of sorting in *T. brucei*.

119 Exploring the secretory pathway in African trypanosomes via proximity labelling

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The endoplasmic reticulum (ER) has been associated with multiple functions to maintain cellular homoeostasis. ER also operates as early secretory organelle, trafficking proteins destined for secretion and is interconnected with varied organelles. *Trypanosoma brucei*, as pathogenic protist, possesses alternative life cycle stages for the mammalian (bloodstream form, BSF) and the insect host (procyclic form, PCF), that secreted various virulence factors for host infection and escape from host immunity, while the mechanism of secretion remains widely uncharacterised. We set out to leverage BioID-mediated proximity protein labelling selecting the ER chaperone luminal binding protein (BiP) linked with an ER retention signal (MDDL) as bait (BiP-BioID-MDDL). Additionally, we employed a truncated BIP construct, (BiPN), lacking the C-terminal ATPase domain and the ER retention signal, endogenously tagged with BioID as secretory reporter (BiPN-BioID), to monitor protein trafficking from ER, over the Golgi to secretion. Streptavidin enrichment proteomics detected 412 proteins in PCF and 506 proteins in BSF, respectively, for the BIP bait. Assignment of functional groups revealed that the majority of these proteins are surface proteins, ER quality control (ERQC) and ER-associated degradation (ERAD) components, and potential virulence factors such as trans-sialidase, metallopeptidase and cysteine peptidase. Consistently, Cy5-streptavidin fluorescence tracing confirmed that the BioID-dependent biotinylation signal in BiP cell lines localises to the ER. BiPN-BioD identified a similar protein cohort that additionally contained early Golgi and Golgi resident proteins such as COPII vesicle coat components and adaptin3. Next, we will trace biotinylated proteins in media supernatants in order to derive a high confidence secretome. Altogether, this study explores the multifaceted roles of the ER in *Trypanosoma brucei*, detailing its involvement in protein modification, quality control, trafficking and secretion of virulence factors.

120 The protein kinase ATAXIA-TELANGIECTASIA AND RAD3-RELATED (ATR) is an important player to guarantee the genome integrity in *Leishmania major*.

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The protein kinase Ataxia-Telangiectasia and Rad3-related (ATR) is a master regulator of the eukaryotic response to DNA injuries that is activated in response to the accumulation of single stranded DNA (ssDNA) and orchestrates checkpoint activation, cell cycle arrest, replication fork stabilization/restart, control of origin firing and telomeric stabilization providing genome maintenance and stability. However, little is known about ATR kinase functions in an organism with a remarkable plastic genome such as *Leishmania*. Using CRISPR/Cas9 editing tool we were able to generate cells expressing a N'terminal tagging (mycATR) that reveals the presence of the kinase at nuclear compartment. The deletion of ATR C'terminal region (mycATRΔC), where the kinase domains are predicted, seems to affect the protein location, expression and/or stability. Those mutant ATR cells showed to be sensitive to replication stress: accumulating ssDNA, DNA damage markers (γH2A), and a disrupted cell cycle. The Marker Frequency Analysis (MFA-seq) showed that in mycATRΔC the replication program changes where some large chromosomes have in average an earlier replication than those ones in mycATR. In addition, in mycATRΔC showed more susceptible to genome variation and accumulates less Single Nucleotide Polymorphisms (SNP) in comparison with in mycATR after replicative stress. Those results suggest that ATR is important to genome maintenance guarantying the proper replication process after stress.

121 TbKAP7/HMG3 is essential for growth and cell division in *Trypanosoma brucei*

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The mitochondrial genome of Trypanosomatids, known as kinetoplast DNA (kDNA), is a large and complex structure that must be faithfully replicated once per cell cycle. The kDNA is associated with small, positively-charged proteins called kinetoplast-associated proteins (KAPs), some of which have demonstrated roles in kDNA compaction and maintenance. TbKAP7 (also known as TbHMG3 due to its C-terminal HMG-box) is a conserved, uncharacterized gene located on chromosome 10 close to related genes encoding TbKAP6 and two isoforms of TbKAP4. Relative to these, the predicted TbKAP7 protein has a short N-terminal extension, although all four have predicted mitochondrial targeting signals. Here we used RNAi-mediated knockdown to show that TbKAP7 is essential for normal growth of both bloodstream and procyclic form *T. brucei*. While effects on kDNA loss and mitochondrial membrane potential were minimal during TbKAP7 knockdown in bloodstream form cells, we observed an accumulation of cells with multiple nuclei and kinetoplasts. In procyclic form *T. brucei*, knockdown of TbKAP7 produces no obvious decrease in minicircle or maxicircle content and no changes in free minicircle replication intermediates. This suggests that TbKAP7, a likely mitochondrial nucleic acid-binding protein, may facilitate cell cycle progression independent of kDNA replication.

122 A novel family of SET domain protein lysine methyltransferases is essential for mitochondrial function in *Trypanosoma brucei*.

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Protein lysine methylation is a ubiquitous post-translational modification catalyzed by SET domain proteins. The trypanosome genome encodes 39 SET domain proteins; nine of which have no identifiable orthologs in other organisms. RNA interference (RNAi) studies show that Tb927.1.2730, Tb927.3.810, Tb927.7.2040 and Tb927.11.13560 are essential to the insect stage, procyclic form (PF) of the parasites and that Tb927.11.13560, which we have named TbSETD, is also essential in bloodstream form parasites. BF RNAi studies with the other SET proteins are in progress. PF TbSETD-deficient cells have altered mitochondrial morphology, increased levels of reactive oxygen species, and increased sensitivity to apoptotic triggers. Despite the absence of a detectable localization sequence, TbSETD localizes to the mitochondria and mitochondrial ribosome proteins and mitoribosome assembly factors comprise most of the TbSETD-binding proteins. Methyl lysine blots show reduced signal of a ~35 kDa protein in RNAi cell lines and recombinant TbSETD reproducibly methylates ~35 kDa and ~ 65 kDa proteins in in vitro assays against fractionated cell lysates. TbSETD Y269A in which the putative catalytic tyrosine is mutated is inactive. We used mass spectroscopy of gel slices to identify potential substrates. These samples contained 13 mitochondrial proteins. Two were mitoribosome assembly factors (Tb927.9.11900, mitoribosomal SSU assembly factor 36 and Tb927.11.5880, mitoribosomal LSU assembly factor 23). The association of TbSETD with mitoribosome and mitoribosome accessory factors and the mitochondrial defects suggest it may play a role in mitoribosome function. The lack of homologs in other organisms suggests a unique role in the biology of these parasites.

123 High-throughput cell-cycle synchronisation of *Leishmania mexicana*

Elizabeth Thwaites (Lancaster University); Chloe Barnes (Lancaster University); Richard Burchmore (Glasgow University); Richard McCulloch (Glasgow University); Michael D. Urbaniak (Lancaster University)

Leishmania mexicana are unicellular parasites with a dixenous lifecycle alternating between a sandfly vector and a mammalian host, causing cutaneous Leishmaniasis in humans. *Leishmania* have an atypical genome organisation, with polycistronic transcription units of functionally unrelated genes, resulting in gene expression that is mostly constitutive and almost entirely regulated post-transcriptionally. How *Leishmania* can regulate their cell cycle in the absence of transcriptional control is not established. Access to high-quality cell-cycle synchronised populations is desirable to enable further study of the regulation of the cell cycle. Centrifugal counter-flow elutriation (CCE) separates a population into fractions based on their size and density, due to two opposing forces of the centrifugal force and the fluid counter-flow. We have shown that CCE is able to produce high-quality, viable populations of G1 cell-cycle synchronised *T. brucei* cells suitable for the study of cell cycle regulation. Here, we present a novel, high-throughput approach to cell cycle synchronisation of promastigote *L. mexicana* using CCE. *L. mexicana* promastigotes, an extracellular form found within the insect vector, display distinct morphological variations as the cells progress through the cell cycle. We applied CCE to cultured *L. mexicana* promastigotes and observed a pattern of eluting cells dissimilar to *T. brucei* due to the wide range of morphologies present. However, we demonstrate that CCE can be used to enrich a G1 population of *L. mexicana* that maintains cell viability and is able to progress through the cell cycle when placed back in culture after elutriation.

124 A triple inducible system to study the role of POLIB in kDNA replication and DNA damage response

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Faithful maintenance of the kinetoplast DNA (kDNA) network is indispensable for survival and life cycle completion of *Trypanosoma brucei*. Persistent gaps (ssDNA regions) are tolerated during kDNA replication suggesting that the DNA damage tolerance response is essential and notably different in Trypanosomatids. *T. brucei* has three paralogs (POLIB, IC and ID) with non-redundant roles in kDNA maintenance however, the division of labor among the paralogs remains unknown. POLIB structure is unique with the exonuclease domain (exo) embedded within the DNA polymerase domain (pol) and is conserved across all kinetoplastid organisms. Recombinant POLIB displays robust Exo activity and produces short extension products while overexpression of a catalytically dead exo variant produces a dominant negative phenotype accumulating linearized minicircles. These data suggest POLIB is not a replicative polymerase but has an essential role in the kDNA damage response. We adapted the Vanillic Acid-Tetracycline (VAT) dual-inducer system for independent and more precisely controlled gene expression in a single cell line for POLIB structure-function studies. POLIBVAT RNAi complementation resulted in sustained and robust expression of wildtype POLIB that rescued the RNAi defects. To induce kDNA specific damage we added cumate inducible elements for expression of mitochondrially targeted damaging agents such as the bacterial toxin DarT to generate double strand breaks. Subsequent work includes characterizing catalytically inactive variants of POLIB to examine the link between kDNA replication and repair, and particularly the role of the POLIB exo activity in clearing linearized minicircles and maxicircles that can accumulate during replication stress and DNA damage.

125 Mechanisms by which African trypanosomes sense and respond to iron availability

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Trypanosoma brucei obtains the essential element iron by uptake of host transferrin (Tf) through its own transferrin receptor (TfR), a heterodimer of the glycoproteins ESAG6 and ESAG7. Under iron starvation conditions, expression of the TbTfR mRNA and protein rapidly increase ~5-fold, resulting in a corresponding increase of uptake of Tf. Using a firefly Luciferase (fLUC) - ESAG6 3'UTR fusion we observed that iron starvation upregulated fLUC activity with a similar magnitude and timing to the upregulation of the transferrin receptor, demonstrating that dynamic regulation is mediated via the 3'UTR (PMID:30596656). Here, we further investigate the mechanisms by which *T. brucei* senses and responds to variation in iron availability. To identify the presence of regulatory motif(s) in the TbTfR 3'UTR we made a series of fLUC reporter cell lines fused with full length or truncated versions of the ESAG6 and ESAG7 3'UTRs. We identified a motif conserved in the 14 BES ESAG6 & ESAG7 genes but absent from the putative chromosome-internal copies. To investigate the mechanism of dynamic regulation of the TbTfR we performed quantitative phosphoproteomic analysis of *T. brucei* cells undergoing iron starvation to identify and quantify the changes in phosphorylation status and protein abundance that occur. Our results suggest a common trans-acting RBP is responsible for dynamic regulation of the TbTfR. We also investigated the conservation, function, and regulation of the 45 putative T. congolense TfRs which have previously been identified by homology, but which are not functionally validated.

126 A NEK protein kinase family member is involved in the differentiation and kinetoplast division of *Trypanosoma cruzi*

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Protein phosphorylation mediates key biological processes in the life cycle of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. The Repressor of Differentiation Kinase 2 (RDK2) belongs to the NEK family, a protein kinase group that is expanded in Trypanosomatid kinomes relative to humans. RDK2 is an essential gene in *Trypanosoma brucei* and *Leishmania mexicana*. In the search of potential targets for antiparasitic interventions, we aimed to study the role of RDK2 in *T. cruzi*. Using immunofluorescence microscopy, an RDK2-3xc-Myc endogenously tagged cell line obtained by CRISPR/Cas9, showed a cytosolic localization for TcRDK2 in epimastigotes, cell derived trypomastigotes, and amastigotes. Our attempts to generate a constitutive TcRDK2 overexpression cell line resulted in non-viable parasites, apparently because high expression levels of the protein lead to cell proliferation arrest and cell death. Subsequently, we generated TcRDK2 tetracycline-inducible mutant cell line and observed impaired epimastigote growth and increased differentiation to metacyclic trypomastigotes upon RDK2 induction of expression. In addition, the ability of tet-induced trypomastigotes to infect host cells was significantly reduced. Furthermore, overexpression of RDK2-K70A, containing an inactivating mutation in the catalytic domain, and ablation of RDK2 in knockout parasites, had no effect in epimastigote growth, but showed a significant decrease of in vitro metacyclogenesis and a higher number of 2N/1K epimastigotes, compared to control cells. Together, our results suggest that RDK2 is involved in metacyclogenesis and kinetoplast division. A phosphoproteomic analysis of epimastigotes overexpressing RDK2 is currently in progress, to identify potential substrates of this protein kinase.

127 Investigating the sequence homology requirements for mosaic VSG formation in *Trypanosoma brucei*

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T. brucei utilizes antigenic variation to evade the host immune system through the switching of its dense Variant Surface Glycoprotein (VSG) coat. The *T. brucei* genome encodes a repertoire of ~2000 VSG-encoding genes, but the majority of these genes are pseudogenes or gene fragments. To expand the usable VSG repertoire and maintain a chronic infection, novel mosaic VSGs can be formed through recombination of two or more VSG genes. Our lab has previously shown that DNA damage can trigger mosaic VSG formation. This process requires sequence homology between the expressed VSG and the donor VSG that is used as a repair template after a DNA break, but little is known about the exact homology requirements for mosaic formation, including the minimum length of homology required for a VSG gene to be recognized as a potential donor sequence. To investigate the homology requirements for mosaic VSG formation, we identified a common recombination site in the VSG Antat1.1 and developed truncated forms of a donor VSG that is commonly utilized around this site, limiting the homology available for donor identification. The truncated constructs were stably integrated into parasites expressing Antat1.1 and double stranded breaks were induced at the common recombination site to promote mosaic VSG formation. We are currently using anchored multiplex PCR sequencing (AMP-seq) to evaluate the minimum sequence homology required to form a mosaic VSG. This approach will provide us with insight into the homology search mechanism used to generate mosaic VSGs.

128 SLAM-seq reveals independent contributions of RNA processing and stability to gene expression in African trypanosomes

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Gene expression is a multi-step process that converts DNA-encoded information into proteins, involving RNA transcription, maturation, degradation, and translation. While transcriptional control is a major regulator of protein levels, the role of post-transcriptional processes such as RNA processing and degradation is less well understood due to the challenge of measuring their contributions individually. To address this challenge, we investigated the control of gene expression in *Trypanosoma brucei*, a unicellular parasite assumed to lack transcriptional control. Instead, mRNA levels in *T. brucei* are controlled by post-transcriptional processes, which enabled us to disentangle the contribution of both processes to total mRNA levels. In this study, we developed an efficient metabolic RNA labeling approach and combined ultra-short metabolic labeling with transient transcriptome sequencing (TT-seq) to confirm the long-standing assumption that RNA polymerase II transcription is unregulated in *T. brucei*. In addition, we established thiol (SH)-linked alkylation for metabolic sequencing of RNA (SLAM-seq) to globally quantify RNA processing rates and half-lives. Our data, combined with scRNA-seq data, indicate that RNA processing and stability independently affect total mRNA levels and contribute to the variability seen between individual cells in African trypanosomes.

129 Quantitative proteomic analysis reveals different responses dependent on the SLAMF1 receptor and the *Trypanosoma cruzi* strain

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Chagas disease is caused by the intracellular protozoan parasite *Trypanosoma cruzi*. This disease affects mainly rural areas in Central and South America, where the insect vector is endemic. However, this disease has become a world health problem since migration has spread to other continents. It is a complex disease with many reservoirs and vectors and a high genetic variability. One of the host proteins involved in the pathogenesis is SLAMF1. This immune receptor acts during the infection of macrophages controlling parasite replication and thus affecting survival in mice but in a parasite strain-dependent manner. Therefore, we studied the role of SLAMF1 by quantitative proteomics in a macrophage in vitro infection and the different responses between Y and VFRA strains of *Trypanosoma cruzi*. We detected different significant up- or down-regulated proteins involved in immune regulation processes, which are SLAMF1 and strain-dependent. Furthermore, independently of SLAMF1, this parasite induces different responses in macrophages to counteract the infection and kill the parasite, as the type I and II IFN responses, NLRP3 inflammasome activation, IL-18 production, TLR7 and TLR9 activation specifically with the Y strain and the IL-11 signaling specifically with the VFRA strain. These results have opened new research fields to elucidate the concrete role of SLAMF1 and discover new potential therapeutic approaches for the Chagas disease.

130 Transcriptomic analysis in macrophages infected with *Trypanosoma cruzi*

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Trypanosoma cruzi is a protozoan parasite responsible for Chagas disease, a significant health burden affecting millions globally. This parasite predominantly infects mammalian host cells, particularly macrophages, eliciting a complex immune response crucial for controlling infection. The interaction between *T. cruzi* and macrophages involves intricate signaling pathways mediated by pattern recognition receptors, which lead to the production of immune mediators. The parasite's evasion strategies, including interference with host cell processes, contribute to chronic infection and dissemination. In this study, we conducted an unbiased analysis of the immune response in mouse macrophages 24 h post-infection with *T. cruzi* using RNA-Seq. Bioinformatics analysis evidenced a key role of Tlr2 and Tlr7 in the immune response against the parasite. We compared the impact on the gene expression of immune mediators using quantitative RT-PCR using two *T. cruzi* strains with different virulence, and WT and Tlr2-/- mouse macrophages. Finally, gene ontology analyses predicted a blockage in iron transport mediated by clathrin and the regulation of the extracellular matrix. Our results shed light on the molecular mechanisms underlying *T. cruzi* infection in macrophages and provide insights into host-parasite interactions mediated by Tlr2. These findings are crucial for identifying novel therapeutic targets and advancing strategies to combat Chagas disease, thus addressing its global public health impact.

131 Inducible Genome-Scale Overexpression and RNAi libraries for Genetic Screens in *Leishmania* species

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The availability of genome-scale genetic screening technologies for *Leishmania* species has been limited. While recent advances in cosmid-based overexpression screens (e.g., Cos-Seq) and systematic CRISPR-Cas9 knockout collections have expanded the options, these platforms lack the versatility of the inducible ORFeome overexpression and RNAi libraries available for the related kinetoplastid *Trypanosoma brucei*. Here we describe our progress in generating the first genome-scale inducible overexpression and RNAi libraries in *Leishmania*. Inducible expression is mediated by conditional site-specific recombination via a split-Cre recombinase (DiCre). There are two iterations of the overexpression platform. First, we took advantage of the availability of a library of ~6800 *T. brucei* open reading frames (i.e., the TbORFeome) cloned into Gateway ENTRY vectors, which enabled efficient transfer of the library into barcoded inducible *Leishmania* expression vectors. *Leishmania* and *T. brucei* share ~6200 orthologous proteins, many of which would be expected to be functionally equivalent. Second, we have applied the lessons learned from the TbORFeome library to our current efforts to generate a Gateway-compatible library of ~8000 of L. donovani ORFs (i.e., the LdORFeome). *L. braziliensis* is one of the few *Leishmania* species with the capacity for RNAi. We will employ a novel method, Strand Displacement Duplication, to generate barcoded, genome-scale, DiCre-inducible stem-loop RNAi libraries from random *L. braziliensis* genomic DNA fragments. For all three library platforms, CRISPR-Cas9 cleavage at a “landing pad locus” will be used to enhance the efficiency of library integration into the rRNA array. Results from a proof-of-principle screen will be discussed.

132 Potential roles for RNA modification in *Trypanosoma brucei* mitochondrial RNA editing

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Kinetoplastid parasites possess unique RNA processing mechanisms that regulate metabolism and development. These include gRNA-directed U-insertion/deletion editing of mitochondrial (mt)-mRNAs. Several multiprotein complexes are required for editing, including RNA-Editing Catalytic, Substrate, and Helicase 2 Complexes (RECC, RESC, REH2C) that bind mRNAs and gRNAs and remodel mRNA sequences. Editing also requires numerous accessory factors including mt-LAF3, a candidate factor we identified in a survey of *T. brucei* mt-RNA modification enzymes. It is pseudouridine synthase (PUS) ortholog and mitoribosome assembly factor. To assess whether mt-LAF3 has additional roles in mt-RNA processing, we generated conditionally null *T. brucei* cells and showed that loss is lethal and disrupts mt membrane potential (ΔΨm). An added mutant gamma-ATP synthase allele permitted ΔΨm maintenance and cell survival. Assessment of the primary effects of mt-LAF3 loss on mt-RNAs revealed decreased 12S and 9S mt-rRNA levels, as expected. Notably, we also observed decreases in mt-mRNA levels, including differential effects on edited vs. pre-edited mRNAs, although surprisingly, mt-LAF3 PUS catalytic activity was not required for these functions. Intriguingly, a human ortholog, RPUSD3, also lacks activity but is essential for mt-RNA pseudouridylation via interaction with catalytic RPUSD4. Thus, another *T. brucei* PUS may be required for mt-RNA editing and processing. While we hypothesize that mt-mRNAs, gRNAs, and rRNAs are PUS substrates, we also present surprising UV Cross-Linking-Immunoprecipitation data that RECC protein KREPB5 directly binds tRNAs. While the functions of tRNAs in RECCs are unclear, it is possible that their modifications may also impact RECC function and editing.

133 Growth and RNA-seq of micropopulations of *T. brucei* in thousands of semi-permeable capsules

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We present an innovative method to grow thousands of micropopulations of *Trypanosoma brucei* at once with their subsequent high throughput sequencing. This method involves encapsulating single *T. brucei* cells in alginate-based semi-permeable capsules (SPCs) allowing the growth of micropopulations from single progenitor cells. SPCs are two-phased structures that consist of aqueous core that is surrounded by a hydrogel shell. This structure doesn't allow cells to escape but facilitates essential for growing nutrient delivery and waste removal, as well as the exchange of small particles such as enzymes and oligonucleotides needed for the following sequencing approaches. Building on the concept of the Split-Pool sequencing method, our technique represents a significant advancement in parasitology. It overcomes the size limitations of growing micropopulations in 96 or 384 well plates. Since each micro population originates from a single cell, it can be treated as a single unit. As the entire micropopulation consists of identical cells, this approach effectively increases the number of reads, thereby enhancing the resolution of sequencing.

134 Identification of cell cycle regulated genes as potential nuclear DNA replication players in *Trypanosoma brucei*

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While the replication fork proteome has been relatively well characterised in model eukaryotes, little work has examined this machinery in Kinetoplastids, including *Trypanosoma brucei*. Homologs of the eukaryotic replicative helicase components (MCM2-7) are present in the parasite, though there is a lack of research detailing their elongation-specific replication dynamics, and no work has asked if and how they interact with the wider replisome during replicative DNA synthesis, including factors that might ensure replication fork fidelity in the face of multigene transcription. In other eukaryotes, MCM2-7 is loaded at origins of replication during G1 phase and travels with the replication fork during S-phase, making its subunits valuable baits for understanding the transition between DNA replication initiation and elongation. Indeed, transcript profiling across the cell cycle suggests that TbMCM2-7 subunit transcripts oscillate, peaking in late G1/S phase, later than expected for pre-replication complex assembly. We are addressing these questions of TbMCM2-7 cell cycle dynamics by endogenously tagging multiple TbMCM2-7 subunits, allowing us to examine their expression and localisation across the cell cycle and to describe the temporal pattern of replisome interactions and replisome dynamics during nuclear DNA replication. Here, we will show initial data on cell cycle expression of TbMCM2-7 subunits, TurboID tagging of some helicase subunits, and expression analysis of wider predicted cell cycle regulated factors that contribute to the replication programme of *T. brucei*.

135 Benzoxaborole AN15368 induces DNA damage and synergizes with DNA repair inhibitors to kill intracellular *Trypanosoma cruzi*

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Chagas Disease is caused by the protozoan parasite *Trypanosoma cruzi* and affects millions of people, mainly in Latin America. The currently available treatments can cause severe side effects and are inconsistently effective in achieving infection cure. Our research group, in collaboration with Anacor Pharmaceuticals, discovered AN15368, a benzoxaborole prodrug that has shown high efficacy in vitro and in vivo, achieving 100% cure rates in non-human primates. AN15368 targets CPSF3, an mRNA-processing endonuclease, suggesting AN15368 primary mechanism of action involves disrupting transcription. We confirmed a rapid loss in active protein synthesis, as measured by L-homopropargylglycine incorporation, after exposure of intracellular *T. cruzi* to AN15368. However, recent studies in mammalian cancer models have shown benzoxaboroles targeting human CPSF3 kill primarily by inducing DNA damage. We report that treatment of intracellular amastigotes with 50 nM AN15368 (~2-5x IC50) for 48 hours resulted in >50% of parasites positive for phosphorylated γH2AX, a sensor for DNA double-strand breaks (DSBs). To explore whether AN15368 may kill via induction of DNA damage, we combined AN15368 with DNA repair inhibitors. Pol-θ inhibitor ART588, RAD51 inhibitor B02, and PARP1 inhibitor Olaparib—all at concentrations which had no effect on parasite growth - each enhanced the activity of AN15368 by ~2-fold (~20 nM to 10 nM). These findings suggest AN15368 initially blocks new protein production via inhibition of mRNA-processing, but DSB accumulation could be its dominant mechanism of killing. Future studies will explore whether DNA repair inhibitors might potentiate AN15368 activity in vivo and allow for shorter courses of treatment.

136 Exploring structural features of a potential fatty acid binding protein of *Leishmania* amazonensis

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Lipids and fatty acids are essential nutrients that Trypanosomatid parasites acquire from their hosts for survival. However, the mechanisms of lipid incorporation remain poorly understood. Fatty acid binding proteins (FABPs) are lipid chaperones that play crucial roles in coordinating lipid trafficking. Bioinformatic searches for FABPs in *Leishmania* genomes identified the conserved LFABP1 gene. The LFABP1 C-terminal domain (LeiFABP) was predicted to be structurally similar to eukaryotic FABPs. Here, we confirmed LFABP1 expression in both life forms of *L. amazonensis* and determined the crystal structure of the LeiFABP domain. Crystallization was performed using the sitting drop method, and X-ray diffraction data were collected at the synchrotron beamline Manacá (Sirius/CNPEM). The structure at 1.8Å resolution, determined with Phenix and CCP4i, confirmed that LeiFABP displays a FABP-like folding, consisting of ten antiparallel beta-strands forming a barrel with a helix-loop-helix motif covering the barrel cavity. Additionally, the crystal structure of LeiFABP exhibits extensive structural homology with FABP members including the vaccine candidate antigen Sm14 from Schistosoma mansoni and EgFABP1 from Echinococcus granulosus. Superimposition of crystallographic structures of LeiFABP and Holo-Sm14 revealed that key residues involved in the interaction with arachidonic acid are conserved and occupy identical positions and orientations in the LeiFABP structure. These residues include Sm14 V25 (LeiFABP-V69), P38 (LeiFABP-P82), S55 (LeiFABP-T100), R78 (LeiFABP-R123), R127 (LeiFABP-R184), and Y129 (LeiFABP-F186). These findings suggest that LFABP1 could play a role in lipid trafficking, which is important for the parasite since *Leishmania*, like other parasitic organisms, have limited capacity for de novo lipid biosynthesis.

137 Unraveling the Enigmatic Feeding Apparatus of *Trypanosoma cruzi*: Using Proximity Labeling to Identify the Molecular Components of the Cytostome-Cytopharynx Complex

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Among the pathogenic Trypanosomatids, *Trypanosoma cruzi* possesses a unique feeding apparatus called the cytostome-cytopharynx complex (SPC), similar to its free-living kinetoplastid relatives. The SPC is essential for endocytic uptake, allowing *T. cruzi* to capture and internalize extracellular material from its host. Despite its central role in bulk-nutrient acquisition, the structural and functional intricacies of the SPC remain largely unexplored, with current knowledge derived primarily from structural studies. Although recent progress has been made, mechanistic studies carried out in our lab have relied primarily on bioinformatic and co-immunoprecipitation-based approaches to identify SPC targeted proteins. Due to the inherent limitations of these approaches, however, we still lack a detailed understanding of the true molecular complexity of this organelle. To enhance our understanding of the SPC's proteomic composition, we implemented a proximity labeling technique using the promiscuous biotin ligase TurboID. We fused TurboID to the myosin-associated protein (MyAP), which is essential for endocytosis and targets the SPC microtubule rootlet fibers. This comprehensive proteomic survey of the MyAP interactome, identified over 100 unique proteins, revealing new insights into the structural and regulatory components of SPC rootlet microtubules. With this validated approach, we are now extending proximity-labeling methods to investigate the sub-proteomes of the SPC pre-oral ridge, cytostome opening, and cytopharynx membrane. Identified targets will be validated through endogenous epitope-tagging and our in-house conditional knockdown system to assess their localization and functional roles in SPC-mediated endocytosis. This work continues to advance our understanding of how SPC-mediated endocytosis functions at the molecular level in *T. cruzi*.

138 Affinity purification of Kinetoplastea mitochondria

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Due to their unique biological features, mitochondria from *Trypanosoma brucei* and *Leishmania* tarentolae have been the focus of extensive research. Here, we introduce a new methodology for purifying intact mitochondria from these organisms. It involves isotonic lysis by nitrogen cavitation, DNA digestion, immunoaffinity purification, proteinase treatment, and sedimentation in a density gradient. The immunoaffinity purification was executed by tagging an outer membrane protein with eGFP and utilizing commercially available Miltenyi magnetic beads. The purity was further enhanced by “shaving” peripheral cytoskeletal proteins with proteinase K prior to gradient centrifugation in OptiPrep media. The isolated organelles exhibited higher purity and integrity than those obtained by current state-of-the-art methods and are suitable for downstream applications, including proteomic analyses and enzymatic activity assays.

139 Elucidating the Organization of Intraflagellar Transport at the Flagellum Base in *Trypanosoma brucei*

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Trypanosoma brucei (*T. brucei*) is the causative agent of African trypanosomiasis in humans and Nagana in cattle. Its flagellum, which fulfills crucial sensory and motility functions through its life cycle and pathogenesis, is assembled and maintained by intraflagellar transport (IFT) protein complexes that traffic bidirectionally along the axoneme. Many details regarding IFT control and regulation in cells remain unknown. Previous research has shown that IFT in *T. brucei* is restricted to a subset of axoneme microtubule doublets (MTDs), unlike in Chlamydomonas reinhardtii, where IFT occurs along all nine MTDs in the axoneme and docking in the flagellum base. Our research aims to elucidate the initiation of IFT restriction in *T. brucei* by investigating its dynamics at the flagellum base. Preliminary studies using cryo-electron tomography (cryo-ET) of focused-ion beam (FIB)-milled procyclic *T. brucei* cells further support that IFT trains are restricted to neighboring MTDs on one side of the axoneme. Subtomogram averaging of the transition zone (TZ) MTDs revealed a unique cryo-EM density feature on the B-tubule, which we used as a marker to show that IFT trains can localize to the A or B tubule. To overcome the challenge in FIB-milling at the flagellum base, we utilized enucleate *T. brucei* mutants (zoids), thin enough for direct cryo-ET acquisition, to characterize the spatial organization of pre-assembled cytoplasmic IFT trains. This study provides valuable insights into the cellular transport processes involved in flagella construction and maintenance in *T. brucei*, highlighting its evolutionary adaptations and unique variations in flagellar function.

140 Cryo-Electron Tomography Analysis of Nuclear Envelope Dynamics During "Closed" Mitosis in *Trypanosoma brucei*

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Trypanosoma brucei is a single-celled eukaryotic parasite that causes trypanosomiasis (African sleeping sickness) in humans and Nagana in cattle in Africa. The cell division machinery in *T. brucei* could be a potential target for new drugs, since this parasite has a unique feature in its mitotic process. Targeting this specific mechanism might lead to the development of selective anti-parasitic therapies with fewer side effects on the host. Unlike many eukaryotes, *T. brucei* undergoes a "closed" mitosis, the nuclear envelope remains intact throughout the process, in contrast to "open" mitosis, where the envelope breaks down. Despite this distinction, the mechanisms of nuclear envelope restructuring during cell division remain poorly understood. Our research aims to elucidate these mechanisms by investigating the morphological and structural changes of the nucleus membrane during closed mitosis. We employ cryogenic electron tomography (cryo-ET) on focused-ion-beam milled procyclic form of *T. brucei* cells, enabling us to visualize cellular ultrastructure in their native state during mitosis. Preliminary data reveal distinct spatial rearrangements of the nuclear envelope. We hypothesize that a nucleus envelope "bridge" forms between the old and new nuclei during closed mitosis. This bridge is driven by spindle microtubules within the nucleus space and supported by vesicles that fused with the nuclear membrane. Our ongoing analysis seeks to uncover the fundamental principles governing this unique form of mitosis and nuclear dynamics, advancing our understanding of *T. brucei* cell division and the biology of parasite proliferation.

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