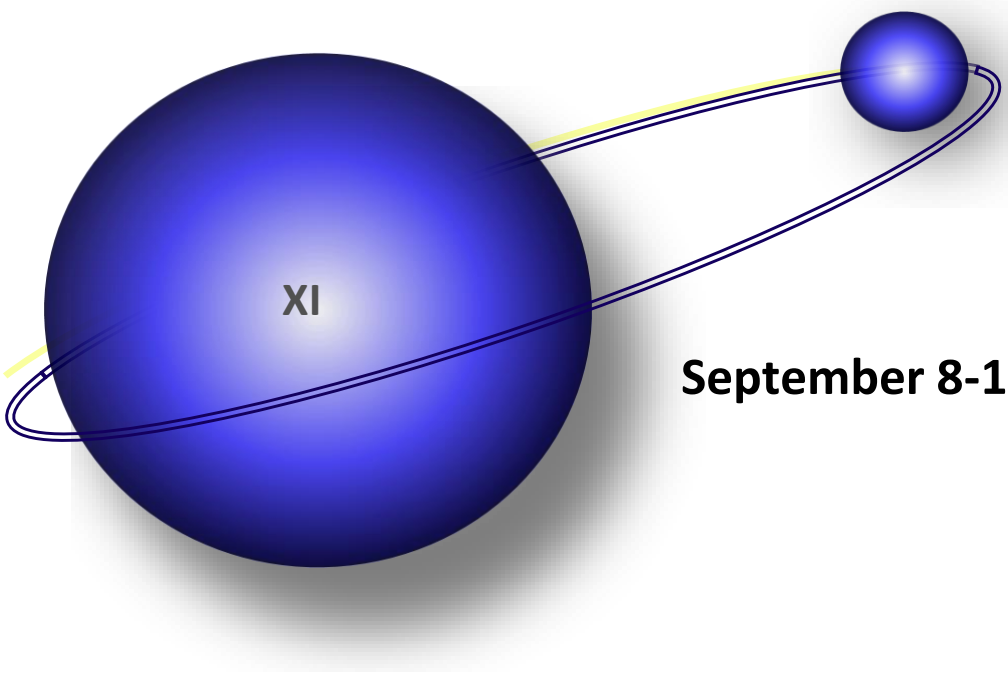
Kinetoplastid Molecular Cell Biology

An International Conference

Marine Biological Laboratory. Woods Hole, MA. USA



September 8-12, 2026

Eleventh Kinetoplastid Molecular Cell Biology Meeting

KMCBM XI

September 8–12, 2026

Marine Biological Laboratory

Woods Hole, Massachusetts, USA

Origins of the KMCB meeting

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 & Luísa M. Figueiredo
2024	Luísa M. Figueiredo, Chris de Graffenried, Megan L. Povelones & Omar S. Harb
2026	Luísa M. Figueiredo, Chris de Graffenried, Megan L. Povelones & Omar S. Harb

Acknowledgments

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 two KMCB speakers to attend the ASTMH 2026 Annual Meeting, to be held November 18 – 22, 2026 at the Gaylord National in National Harbor, Maryland (adjacent to Washington, DC).

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 8, 2026		
2:00PM – 5:00PM	Arrival and registration	Swope
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:05PM	Session I: Gene regulation	Clapp
9:00PM – 11:00PM	Mixer	Swope

Wednesday, September 9, 2026		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 11:40AM	Session II: RNA editing special 40th anniversary session	Clapp
12:00PM – 1:30PM	Lunch	Swope
2:00PM – 5:20PM	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 10, 2026		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 12:10PM	Session IV: Biochemistry	Clapp
12:15PM – 1:30PM	Lunch	Swope
1:45PM – 5:00PM	Free time	
5:00PM – 6:30PM	Dinner	Swope
7:00PM – 9:00PM	Poster Session B	Swope
9:00PM – 11:00PM	Mixer and posters continued	Swope

Friday, September 11, 2026		
7:30AM – 8:30AM	Breakfast	Swope
8:45AM – 11:30AM	Session V: Treatments and vaccines	Clapp
12:00PM – 1:30PM	Lunch	Swope
2:00PM – 5:00PM	Session VI: The host perspective	Clapp
5:30PM – 7:00PM	BBQ Dinner	Meig's Room
7:00PM – 10:00PM	Mixer	

Saturday, September 12, 2026		
7:30AM – 8:30AM	Breakfast	Swope
9:00AM	Depart	

RNA editing 40th anniversary

While various forms of post-transcriptional RNA editing are now recognized across eukaryotic domains, the phenomenon was first brought to light forty years ago in trypanosomes with the startling discovery of uridine insertion and deletion. The story of RNA editing began in 1986, when the Benne lab showed that the mitochondrial COXII transcript of *Trypanosoma brucei* contained four uridines that were not encoded in the DNA, restoring the correct reading frame. This unexpected observation led to the concept of **RNA editing**. One year later, the Stuart lab showed that uridine addition also occurred in cytochrome b transcripts and, importantly, that it was developmentally regulated. In 1988, work from the Stuart and Simpson labs revealed that editing involved both uridine insertion and deletion and could generate conserved protein-coding sequences as well as AUG initiation codons. Later, the Simpson, Stuart, and Sollner-Webb labs described the role of guide RNAs, the editosome, and the precise step-by-step catalytic process of RNA editing. Over the following decades, many laboratories around the world joined the field, uncovering even more amazing details of the molecular machinery, mechanisms, regulation and biological significance of RNA editing. Their collective work transformed an unexpected observation in trypanosome mitochondria into a rich and influential field of RNA biology. The discovery of RNA editing in trypanosomes challenged the central dogma and paved the way for scientists investigating other forms of RNA modification, guide RNAs, macromolecular enzymatic machines, and RNA-targeted therapeutics.

Benne, R., Van den Burg, J., Brakenhoff, J. P. J., Sloof, P, Van Boom, J. H. & Tromp, M. C. (**1986**) Major transcript of the frameshifted coxll gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA, Cell 46, 819–826.

Feagin, J. E., Jasmer, D. P. & Stuart, K. (**1987**) Developmentally regulated addition of nucleotides within apocytochrome b transcripts in Trypanosoma brucei, Cell 49, 337–345.

Shaw, J. M., Feagin, J. E., Stuart, K. & Simpson, L. (**1988**) Editing of kinetoplastid mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons, Cell 52, 401–411.

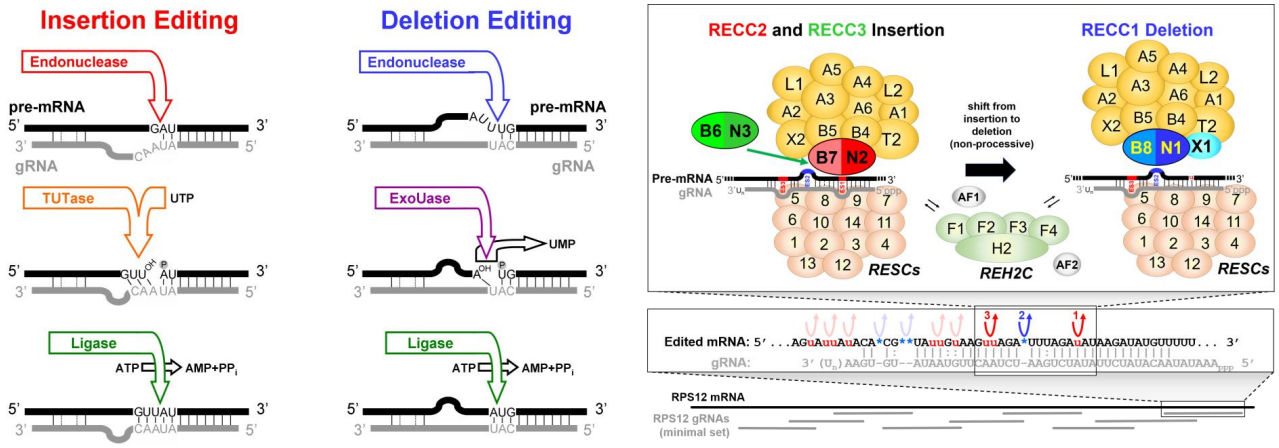


Image courtesy of James Carnes, Seattle Childrens Research Institute

Talks

I. Gene regulation

Tuesday, September 8, 2026 · 7:00PM – 9:05PM
Chair: Noelia Lander

1. Molecular and structural dissection of VEX2 rewrites eukaryotic rules of helicase regulation to drive monogenic VSG expression
Leon Walther[1], Hideharu Hashimoto[2], Erik W. Debler[2], Michele Tinti[3], David Horn[3], Jamie N. Blaza[1], Chris H. Hill[1], Joana R. C. Faria[1]
[1] *University of York*
[2] *Thomas Jefferson University*
[3] *University of Dundee*

2. A fine scale single-cell roadmap of the cell cycle and metacyclogenesis in *Trypanosoma cruzi*
Caroline D Keroack[1], Eric D Salomaki[1], Sophie Marcus-Wade[1], Madeline F Malfara[1], Jacqueline Lopez[1], Erin M Kennedy[1], Christopher L de Graffenried[1]
[1] *Brown University*

3. DAC4-dependent chromatin loops coordinate efficient transcription in *Leishmania*
Manoj Koirala[1], Hanna Campbell-Irwin[1], Lissa Cruz-Saaverdra[1], Suellen Maran[2], Nilmar Moretti[2], Igor Cestari[1]
[1] *McGill University*
[2] *Universidade Federal de São Paulo*

4. An in vitro system to dissect antigen diversification in *Trypanosoma brucei* reveals donor template fate and selection requirements
Jaclyn E Smith[1], Kevin J Wang[2], Erin M Kennedy[2], Monica R Mugnier[2]
[1] *University of Maryland Baltimore*
[2] *Johns Hopkins School of Public Health*

5. *Trypanosoma brucei* RNase H2 uses a non-canonical PIP motif to interact with the PCNA sliding clamp
Manon Andreck[1], Hideharu Hashimoto[1], Erik W Debler[1]
[1] *Thomas Jefferson University*

6. TbRAP1 binds to selected RNAP II transcription termination sites to block RNAP II from reading through RNAP III-transcribed genes
Marjia Afrin[1], Lovlesh Thakur[1], Farheen Naz[1], Prem Kushwaha[1], Amit Gaurav[1], Jillian Gady[1], Bibo Li[1]
[1] *Cleveland State University*

Turbo Talks

P47. Investigating the antigenic diversity of *T. brucei* VSG mosaics to study the evolution of host-pathogen interactions

Caterina Canetta[1], Monique van Straaten[1], Sandra Gonçalves Trindade[2], Abdubasit Amin[3], Jaclyn Smith[4], Adrien Jolly[5], Luísa M. Figueiredo[2], Monica Mugnier[4], Nina Papavasiliou[1], Erec Stebbins[1]

[1] *DKFZ*

[2] *GIMM*

[3] *University of Ilorin*

[4] *Johns Hopkins Bloomberg School of Public Health*

[5] *Goethe University Frankfurt, DKFZ*

P48. Chromatin Profiling Unraveled: Comparing ATAC-seq Profiles in Monomorphic versus Pleomorphic *Trypanosoma brucei*

Ruth Shelton[1], Keith Matthews[1]

[1] *University of Edinburgh*

P49. Novel elements of translation termination in trypanosomatids

Julie Kovarova[1], Vojtech Cerny[1], Nathalia Ballesteros Chitiva[1], Zdenek Paris[1]

[1] *Institute of Parasitology*

P50. Understanding Recombination Mechanisms Within Transcriptionally Silent Variant Surface Glycoproteins (VSGs) in *Trypanosoma brucei*

Zachary E. Brown[1], Monica R. Mugnier[1], Jaclyn E. Smith[2], Erin M. Kennedy[2]

[1] *Johns Hopkins Bloomberg School of Public Health*

[2] *Johns Hopkins Bloomberg School of Public Health; University of Maryland School of Medicine (present)*

P51. Investigating TERRA functions in TbTRF-mediated telomere maintenance in *Trypanosoma brucei*

Lovlesh Thakur[1], SK Abdus Sayeed[1], Bibo Li[1]

[1] *Cleveland State University*

P52. Poly(A) polymerases and the dynamic regulation of VSG mRNA in *Trypanosoma brucei*

Christoph Wenzl[1], Sheryll Ashu[2], Christian Schmidt[3], Michal Malecki[1], Luísa M. Figueiredo[1]

[1] *Gulbenkian Institute for Molecular Medicine*

[2] *Technische Universität Berlin*

[3] *Universität Regensburg*

II. RNA editing special 40th anniversary session

Wednesday, September 9, 2026 · 8:45AM – 11:40AM

Chair: Jason Carnes

7. RNA editing: An introduction

Jason Carnes[1]

[1] *Seattle Childrens Research Institute*

8. KREH3 helicase couples RNA processing and editing in trypanosome mitochondria

Takuma Suematsu[1], Xiaojing Zhao[2], Andres F. Vacas[1], Chenyu Fan[2], Clinton Yu[3], Md Solayman[1], Lan Huang[3], Liye Zhang[2], Ruslan Afasizhev[1], Inna Afasizheva[1]

[1] *Boston University*

[2] *ShanghaiTech University*

[3] *University of California, Irvine*

9. Tandem zinc-fingers of an essential RNA helicase cofactor KH2F1 differentially impact editing of mitochondrial transcripts in *Trypanosoma brucei*

Suzanne M McDermott[1], Cody Goode[2], Tyler Rodshagen[1], Sunil K Sharma[2], Joshua Meehan[2], Alaistair Ivens[3], Nicholas J Savill[3], Achim Schnaufer[3], Jorge Cruz-Reyes[2]

[1] *Seattle Childrens Research Institute*

[2] *Texas A&M University*

[3] *University of Edinburgh*

10. Extensive developmental regulation of COX3 Editing Is Driven by Reversible REH2C-Dependent ter-gRNAs in Trypanosome brucei

Cody Goode[1], Sunil K Sharma[1], Tyler Rodshagen[2], Joshua Meehan[1], Alaisdair Ivens[3], Nicholas J Savil[3], Achim Schnaufer[3], Suzanne M McDermott[2], Jorge Cruz-Reyes[1]

[1] *Texas A&M University*

[2] *Seattle Children's Research Institute*

[3] *University of Edinburgh*

11. The KREPA3 L270 domain functions in gRNA selection and utilization during U Indel RNA editing in *Trypanosoma brucei*

Jason Carnes[1], Tyler Rodshagen[1], Maxwell Tracy[1], Brittney Davidge[1], Suzanne M. McDermott[1], Kenneth Stuart[1]

[1] *Seattle Children's Research Institute*

COFFEE BREAK

12. Three-dimensional topology of kinetoplast DNA revealed by cryoET

Yun-Tao Liu[1], Jonathan Jih[1], Andres Vacas[2], Yang Huang[1], Katherine Zhang[1], Md Solayman[2], Inna Afasizheva[2], Ruslan Afasizhev[2], Z. Hong Zhou[1]

[1] *University of California, Los Angeles*

[2] *Boston University*

13. Cell cycle regulated kinetoplast proteins regulate kDNA replication, segregation and cell cycle progression in *Trypanosoma brucei*

Max Pendlebury[1], Michaela Svobodová[1], Vyacheslav Yurchenko[2], Ignacio Durante[1], Julius Lukeš[1], Michael Hammond[1]

[1] *Institute of Parasitology, Biology Centre*

[2] *Life Science Research Centre, University of Ostrava*

14. Control of Minicircle replication by *Trypanosoma brucei* PIF1

Cara M Jenkins[1], Raveen Armstrong[1], Sherlyn Alex[1], Michele M Klingbeil[1]

[1] *University of Massachusetts*

Turbo Talks

P53. KREH4 Emerges as a Novel Member of the Mitochondrial RNA Helicase Network in *Trypanosoma brucei*

Yingzhe Mark Xia[1], Takuma Suematsu[1], Andres Vacas[1], Inna Afasizheva[1]

[1] *Boston University*

P54. Reconstitution and interaction mapping of RNA editing catalytic complexes in *Trypanosoma brucei*

Dmitrii Afonin[1], Clinton Yu[2], Takuma Suematsu[1], Andres Vacas[1], Lan Huang[2], Inna Afasizheva[1], Ruslan Afasizhev[1]

[1] *Boston University*

[2] *University of California*

P55. Substrate recognition in RNA editing may involve combinatorial interactions between dsRBDs and ZnFs in *Trypanosoma brucei*development.

Lars O'Hara[1], Cody Goode[1], Tyler Rodshagen[2], Suzanne M McDermott[3], Jorge Cruz-Reyes[1]

[1] *Department of Biochemistry and Biophysics, Texas A&M University*

[2] *Center for Global Infectious Disease Research, Seattle Children's Research Institute*

[3] *Center for Global Infectious Disease Research, Seattle Children's Research Institute. Department of Pediatrics, University of Washington School of Medicine*

P56. Networks of *Trypanosoma brucei* mitochondrial protein and RNA interactions revealed by CXMS and hiCLIP

Tyler Rodshagen[1], Cody Goode[2], Jorge Cruz-Reyes[2], Suzanne M McDermott[1]

[1] *Seattle Childrens Research Institute*

[2] *Texas A&M University*

P57. Disruption of a key regulatory editing complex profoundly affects cell growth, editing regulation, and editosome-holoenzyme assembly during development

Sunil K Sharma[1], Cody Goode[1], Tyler Rodshagen[2], Joshua Meehan[1], Alaisdair Ivens[3], Nicholas J Savill[3], Achim Schnaufer[3], Suzanne M McDermott[2], Jorge Cruz-Reyes[1]

[1] *Texas A&M University*

[2] *Seattle Children's Research Institute*

[3] *University of Edinburgh*

P58. Structural basis of the RNA editing cascade in trypanosome mitochondria

Yun-Tao Liu[1], Andres Vacas[2], Jonathan Jih[1], Xiaojing Zhao[3], Clinton Yu[4], Jane K.J. Lee[1], Takuma Suematsu[2], Md Solayman[2], Hong Wang[2], Xiaorong Wang[4], Lan Huang[4], Liye Zhang[3], Inna Aphasizheva[2], Z. Hong Zhou[1], Ruslan Aphasizhev[2]

[1] *University of California, Los Angeles*

[2] *Boston University*

[3] *ShanghaiTech University*

[4] *University of California, Irvine*

III. Cell biology

Wednesday, September 9, 2026 · 2:00PM – 5:20PM
Chair: Galadriel Hovel-Miner

Plenary Talk

15. The dynamic intracellular world of *Trypanosoma cruzi*

John M Kelly[1]

[1] *Department of Infection Biology, London School of Hygiene and Tropical Medicine, UK*

16. Unraveling the Role of Proteolytic Pathways in *Trypanosoma cruzi* Differentiation and Egress

Sara De Grandis[1], Delia Bogenstätter[1], Luca Kissling[1], Gaëlle Lentini[1]

[1] *Institute of Cell Biology, University of Bern*

17. Elucidating the in situ Spatial Organization of Intraflagellar Transport (IFT) at the Flagellar Base in *Trypanosoma brucei*

Sophia R. Staggers[1], Mingqi Zhao[1], Oliver Harrigan[2], Sabrina Absalon[2], Yameng Huang[3], Zhangyu (Sharey) Cheng[4], Yongxin (Leon) Zhao[4], Muyuan Chen[5], Stella Y. Sun[1]

[1] *University of Pittsburgh School of Medicine*

[2] *Indiana University School of Medicine*

[3] *National University of Singapore*

[4] *Carnegie Mellon University Mellon College of Science*

[5] *Stanford University SLAC National Accelerator Laboratory*

18. A Flagellar Membrane cAMP-phosphodiesterase in *Leishmania mexicana* is Critical for Colonization of the Sand Fly

Judy VanSlyke[1], Marco A Sanchez[1], Eva Iniguez[2], Tiago Serafim[2], Shaden Kamhawi[2], Scott M Landfear[1]

[1] *Oregon Health & Science University*

[2] *Vector Molecular Biology Section, National Institute of Allergy and Infectious Diseases*

19. FLAM6 is a cAMP-binding protein that controls flagellar length, host cell infection, and vector colonization in *Trypanosoma cruzi*

Asima Das[1], Riley Hunter[1], Syeda Farjana Hoque[1], Joshua B Benoit[1], Miguel A Chiurillo[1], Noelia Lander[1]

[1] *University of Cincinnati*

COFFEE BREAK

20. *Trypanosoma cruzi* SPC-mediated endocytosis serves as a redundant, dynamically regulated mechanism for heme acquisition

Gonzalo Seminario-Mondejar[1], Ruby Harrison[2], Menna Etheridge[1], Ronald Drew Etheridge[1]

[1] *University of Georgia*

[2] *University of Akron*

21. Investigating the Role of TcCIF1 Throughout the Life Cycle of *Trypanosoma cruzi*

Juliana Nunes Roson[1], Cesar González-Zermeño[1], Miguel Chiurillo[1]

[1] *University of Cincinnati*

22. Dynamics within the *Trypanosoma brucei* subpellicular microtubule array

Laura A Smithson[1], Ana Sofia Cepeda Diaz[1], August Guang[1], Eleni Hughes[2], Anja Schmidt[2], Jesse Aaron[2], Christopher L de Graffenried[1]

[1] *Brown University*

[2] *Advanced Imaging Centre, Janelia Research Campus*

Turbo Talks

P59. Functional characterization of the transmembrane protein 18 (TMP18) in *Leishmania mexicana*

Kate Sagoe[1], Andreu Saura[1], Alexandra Zakharova[1], Vyacheslav Yurchenko[1]

[1] *Ostrava University*

P60. Using proteomics to uncover clues for RDK1 function in *Trypanosoma brucei*

Ashutosh P Dubey, Parul Pandey, Dinh Bui, Chukwujekwu Aleke, Joseph Smith

P61. In situ cryo-ET reveals RP2-dependent organization of intraflagellar transport and early flagellum assembly in *Trypanosoma brucei*

Mingqi Zhao[1], Sophia R. Staggers[1], Stella Y. Sun[1]

[1] *University of Pittsburgh*

P62. Exploring the Role of Major Surface Proteases in *T. brucei* Infection

Sophie Marcus-Wade[1], Jane Dias[1], Sandra Trindade[1], Catarina Gadelha[2], Bill Wickstead[2], Luísa M. Figueiredo[1]

[1] *Gulbenkian Institute for Molecular Medicine*

[2] *University of Nottingham*

P63. Deciphering the role of B cells in Chronic Chagas Cardiomyopathy pathogenesis

Shweta Deshpande[1], Carolina Duque[1], Sergio Gómez[2], Julio Mantilla[3], Paula Baustista[2], Lyda Rojas[2], Luis Echeverria[2], Martha Diaz[3], Robert Gilman[1], Monica Mugnier[1]

[1] *Johns Hopkins University*

[2] *Fundación Cardiovascular de Colombia*

[3] *Universidad Industrial de Santander*

IV. Biochemistry

Thursday, September 10, 2026 · 8:45AM – 12:10PM

Chair: Stella Sun

Plenary Talk

23. ATP Synthase as an architect of mitochondrial remodeling

Michaela Husová[1], Oskar Lipinski[2], Rachel Gerova[3], Tomáš Skalický[2], Tomáš Bílý[2], Alexey Bondar[2], František Kitzberger[2], Marc Field[4], Martin Zoltner[5], Richard Wheeler[6], Bill Wickstead[3], Catarina Gadelha[3], Alena Zíková[2]

[1] *Faculty of Science, USB*

[2] *Biology Centre, CAS*

[3] *School of Life Sciences, University of Nottingham*

[4] *School of Life Sciences, University of Dundee*

[5] *Faculty of Science, BIOCEV*

[6] *School of Biological Sciences, University of Edinburgh*

24. Lineage-specific innovations expand mitochondrial proteomes in kinetoplastids

Andres F. Vacas[1], Md Solayman[1], Sarah E. Calvo[2], Khanh Nguyen[3], Cameron Genxuan Lian[3], Keith D. Rivera[3], Michael Z. Chen[2], Namrata D. Udeshi[3], Vamsi K. Mootha[2], Ruslan Afasizhev[1], Inna Afasizheva[1]

[1] *Boston University*

[2] *Massachusetts General Hospital*

[3] *Broad Institute*

25. Conserved and divergent mitochondrial assemblies in kinetoplastid parasites

Md Solayman[1], Yi-Ting Liao[2], Sarah E. Calvo[2], Andres F. Vacas[1], Malvina Papanastasiou[3], Keith D. Rivera[3], DR Mani[3], Brian W. Boyle[3], Felicia G. Deng[2], Namrata D. Udeshi[3], Vamsi K. Mootha[2], Inna Afasizheva[1], Luke H. Chao[2], Ruslan Afasizhev[1]

[1] *Boston University*

[2] *Harvard Medical School*

[3] *Broad Institute*

26. Influence of oxidative stress on the metabolic remodeling of *Trypanosoma cruzi* during the initial stages of cellular infection

Michel A Silva[1], Mario A Izidoro[2], Mirella Aricó[1], Bruno Souza Bonifácio[1], Adriana Corrêa da Silva[1], Leonardo Loch[1], Adriana Degrossoli[3], Luiz Juliano[1], Sergio Schenkman[1]

[1] *Universidade Federal de São Paulo*

[2] *Hospital São Paulo*

[3] *Universidade Federal de Lavras*

27. VEuPathDB.org: bioinformatic tools for genomic-scale data exploration, analysis, integration & discovery

Omar S Harb[1], David S Roos[1], on behalf of the VEuPathDB team

[1] *University of Pennsylvania*

COFFEE BREAK

28. Phospholipases as Essential Mediators of Lipid Remodeling in *Leishmania*

Salma Waheed Sheikh[1], Somrita Basu[1], Neha Biyani[1], Kai Zhang[1]

[1] *Texas Tech University*

29. A reversible mesocyclic-like developmental state of *Trypanosoma brucei* is regulated by tryptophan-dependent nutrient sensing

Eva Doleželová[1], Hana Pavlisková[2], Tomáš Skalický[1], Martin Moos[1], Vladimír Varga[2], Alena Zíková[1]

[1] *Biology Centre, CAS*

[2] *Institute of Molecular Genetics, CAS*

30. Visualization of Apolipoprotein L1 at the plasma membrane of African Trypanosome: primary role in cell death

Kayla Leiss[1], Arva Demaliaj[1], Sara Fresard[1], Jayne Raper[1]

[1] *Hunter College*

Turbo Talks

P68. Specific metabolic changes in host cells are required to induce dormancy in *Trypanosoma cruzi*

Leonardo Loch[1], Talícia dos Santos Silva[2], Paula Rego Barros Bittencourt-Cunha[1], Michel Augusto Silva[1], Carolina Borsoi de Moraes[2], Sergio Albuquerque[2], Sergio Schenkman[1]

[1] *Universidade Federal de São Paulo*

[2] *Universidade de São Paulo*

P69. Exploration of Phosphatidylcholine Remodeling in *Leishmania major*

Noah Mueller[1], Kai Zhang[1]

[1] *Texas Tech University*

P70. Ubiquitin-Conjugating Enzymes in *Leishmania infantum*

Olivia Baqui Damário Gomes[1], Caroline Torres[2], Giovanna da Costa Mancin[3], Lucas Henrique de Mello Facione[3], Izaira Tincani Brandão[3], Munira Muhammad Abdel Baqui[3], Felipe Roberti Teixeira[4], Marcelo Damário Gomes[3]

[1] *Faculty of Medicine of Catanduva; Federal University of Sao Carlos*

[2] *School of Medicine of Ribeirao Preto; Federal University of Sao Carlos*

[3] *School of Medicine of Ribeirao Preto*

[4] *Federal University of Sao Carlos*

P71. REDOX Biosensors for Analysis of Drug Effects on Bloodstream Form *Trypanosoma brucei*

Luiz Lola Pereira[1], Nyhal Metidji[1], Penny Lee[1], Galadriel Hovel-Miner[1]

[1] *Stony Brook University*

P72. SHARK-mediated knockdown of *Trypanosoma cruzi* Polo-like kinase (PLK) and Flagellar Pocket-Associated Cytoskeletal Components

Madeline F Malfara[1], Caroline Keroack[1], Jacqueline Lopez[1], Sophie Marcus-Wade[1], Christopher de Graffenried[1]

[1] *Brown University*

V. Treatments and vaccines

Friday, September 11, 2026 · 8:45AM – 11:30AM

Chair: Joseph Smith

31. Glycosomal compartmentalisation and peroxin-mediated resistance to Allopurinol in *Leishmania infantum*

Fereshteh Fani[1], Philippe Leprohon[1], Marc Ouellette[1]

[1] *Centre de recherche en infectiologie du CHU de Québec*

32. Genome-Scale Inducible RNAi library Screens in *Leishmania braziliensis*

Corinne M Fargo[1], Sloane M Domico[1], Stephen M Beverley[2], Phillip A Yates[1]

[1] *Oregon Health & Science University*

[2] *Washington University School of Medicine*

33. The *T. brucei* Inducible Expression Library: Method Updates & Identification of Novel Nitroaromatic Resistance Genes

Penny Lee[1], Kendal Faussete[1], Anushka Shome[1], Elaine Mei[1], Allan Li[1], Nicole Hooshiari[1], Franck Dumetz[1], Galadriel Hovel-Miner[1]

[1] *Stony Brook University*

34. Disruption of the DOT1A-RNaseH2 Interaction for Anti-Parasitic Therapy

Anna Paulson[1], Hideharu Hashimoto[1], Manon Andreck[1], Victoria S. Frisbie[1], Janna Kiselar[2], Laura Hartleb[3], Christian J. Janzen[3], Erik W. Debler[1]

[1] *Thomas Jefferson University*

[2] *Case Western Reserve University*

[3] *University of Würzburg*

COFFEE BREAK

35. A genome wide antigen-antibody screen identifies a yeast-based therapeutic vaccine candidate for Chagas disease

Mira Loock[1], Lissa Cruz-Saavedra[1], Valeria Bernal Araujo[1], Igor Cestari[1]

[1] *McGill University*

36. Genomic and transcriptomic profiling of intracellular amastigotes implicates membrane lipid remodeling in amphotericin B tolerance in *Leishmania* (Viannia) *braziliensis*

Maria C. Echeverry[1], Carlos A. Clavijo[1], Jorge A. Duitama[2], Visnu Chaparro[3], Maria P. Cadena[1], Maritza Jaramillo[3]

[1] *Universidad Nacional De Colombia*

[2] *Universidad de los Andes*
[3] *INRS-Centre Armand Frappier Sante Biotechnologie*

37. Comparative genetic validation of *Trypanosoma cruzi* proline pathway enzymes as potential drug targets

Ana C. Mengarda[1], Janaina de F. Nascimento[2], Maria B. Cassera[3], Ronald D. Etheridge[3], Ariel M. Silber[2]

[1] *University of São Paulo/University of Georgia*

[2] *University of São Paulo*

[3] *University of Georgia*

38. Role of protein kinases in paromomycin resistance in *Leishmania infantum*

Raouia Fakhfakh[1], Jaideep Mallick[1], Arijit Bhattacharya[1], Adriano Coelho[1], Philippe Leprohon[1], Marc Ouellette[1]

[1] *Centre de recherche en infectiologie du CHU de Québec et département de microbiologie et immunologie, Faculté de médicine, université Laval*

VI. The host perspective

Friday, September 11, 2026 · 2:00PM – 5:00PM
Chair: Suzanne McDermott

Plenary Talk

39. ECLIPSE: An intervention programme to improve patient journey and reduce stigma for people with cutaneous leishmaniasis in Brazil, Ethiopia and Sri Lanka

Helen P Price (on behalf of the ECLIPSE team)[1]

[1] *School of Life Sciences, Keele University, Newcastle-under-Lyme, United Kingdom*

40. A genome-wide yeast surface display screen to identify *Trypanosoma cruzi* proteins involved in host cardiomyocyte infection

Lissa Cruz-saavedra[1], Mira Loock[1], Igor Cestari[1]

[1] *Mcgill University*

41. Phenotypic and genomic adaptation to in vitro culture of a novel *Trypanosoma cruzi* strain isolated from a cardiomyopathy patient

Jill Hakim[1], Sneider Gutiérrez[1], Carolina Duque[1], Dharani Moorthy[1], Jaclyn Smith[2], David Serre[2], Robert Gilman[1], Monica Mugnier[1]

[1] *Johns Hopkins*

[2] *University of Maryland*

42. *Trypanosoma cruzi* Infection and its Extracellular Vesicles Induce Host Nuclear Remodeling and DNA Damage Responses

Raul Alexander Gonzales-Cordova[1], Lucas Henrique Melo Falcione[1], Mayla Gabriela Zanchetta Venancio[1], Camila Gachet-Castro[1], Berta Barnadas Carceller[2], Carmen Fernández Becerra[2], Munira Muhammad Abdel Baqui[1]

[1] *Ribeirao Preto Medical School, University of Sao Paulo*

[2] *Medical School, University of Barcelona*

COFFEE BREAK

43. Parasite-Mediated Host and Vector Modulations underpinning Livestock Trypanosomiasis Transmission

Simon Kiplimo Tawich[1], Merid Negash Getahun[1], Christopher W Weldon[2], Souleymane Diallo[1], John Ngiela[1], Antoine Barreaux[1], Steeven Belvinos[1], Chiro Rungua[1], Victor Ndunyu[1], Daniel Masiga[1]

[1] *International Centre of Insect Physiology and Ecology (ICIPE)*

[2] *University of Pretoria*

44. From One Protease to Two Functions: Genome Plasticity and Visceral Adaptation in *Leishmania donovani*

Debolina Manna[1], Sunandini Bhattacharya[1], Shounak Jana[1], Kristína Záhonová[2], Suvendu Nandi[1], Deneshraj Srinivasan[1], Nilanjan Pradhan[1], Nidhi Pandey[1], Debarati Biswas[1], Mahitosh Mandal[1], Gayatri Mukherjee[1], Vyacheslav Yurchenko[2], Budhaditya Mukherjee[1]

[1] *School of Medical Science and Technology, Indian Institute of Technology Kharagpur*

[2] *Life Science Research Centre, Faculty of Science, University of Ostrava*

45. GT15 is required for *Trypanosoma congolense* cytoadhesion

Marta Tiago[1], Leonor Loira[1], José Carlos Franco[2], Amar Dhanjal[2], Eduardo Esteves[1], Bill Wickstead[3], Catarina Gadelha[3], Luísa M. Figueiredo[4], Álvaro Acosta-Serrano[2], Sara Silva Pereira[1]

[1] *Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa*

[2] *College of Science, University of Notre Dame*

[3] *School of Life Sciences, University of Nottingham*

[4] *Gulbenkian Institute for Molecular Medicine, Faculdade de Medicina da Universidade de Lisboa*

46. Resolving the Transcriptional Landscape of *Trypanosoma brucei* Adipose Tissue Forms at the Single-Cell Level

Jaiganesh Jagadeesh[1], Lara Lopez-Escobar[1], Abdulbasit Amin[1], Michal Malecki[1], Luísa M. Figueiredo[1]

[1] *Gulbenkian Institute for Molecular Medicine*

Poster Session A

Wednesday, September 9, 2026 · 7:00PM – 9:00PM

P47. Investigating the antigenic diversity of *T. brucei* VSG mosaics to study the evolution of host-pathogen interactions

Caterina Canetta[1], Monique van Straaten[1], Sandra Gonçalves Trindade[2], Abdubasit Amin[3], Jaclyn Smith[4], Adrien Jolly[5], Luísa M. Figueiredo[2], Monica Mugnier[4], Nina Papavasiliou[1], Erec Stebbins[1]

[1] *DKFZ*

[2] *GIMM*

[3] *University of Ilorin*

[4] *Johns Hopkins Bloomberg School of Public Health*

[5] *Goethe University Frankfurt, DKFZ*

P48. Chromatin Profiling Unraveled: Comparing ATAC-seq Profiles in Monomorphic versus Pleomorphic *Trypanosoma brucei*

Ruth Shelton[1], Keith Matthews[1]

[1] *University of Edinburgh*

P49. Novel elements of translation termination in trypanosomatids

Julie Kovarova[1], Vojtech Cerny[1], Nathalia Ballesteros Chitiva[1], Zdenek Paris[1]

[1] *Institute of Parasitology*

P50. Understanding Recombination Mechanisms Within Transcriptionally Silent Variant Surface Glycoproteins (VSGs) in *Trypanosoma brucei*

Zachary E. Brown[1], Monica R. Mugnier[1], Jaclyn E. Smith[2], Erin M. Kennedy[2]

[1] *Johns Hopkins Bloomberg School of Public Health*

[2] *Johns Hopkins Bloomberg School of Public Health; University of Maryland School of Medicine (present)*

P51. Investigating TERRA functions in TbTRF-mediated telomere maintenance in *Trypanosoma brucei*

Lovlesh Thakur[1], SK Abdus Sayeed[1], Bibo Li[1]

[1] *Cleveland State University*

P52. Poly(A) polymerases and the dynamic regulation of VSG mRNA in *Trypanosoma brucei*

Christoph Wenzl[1], Sheryll Ashu[2], Christian Schmidt[3], Michal Malecki[1], Luísa M. Figueiredo[1]

[1] *Gulbenkian Institute for Molecular Medicine*

[2] *Technische Universität Berlin*

[3] *Universität Regensburg*

P53. KREH4 Emerges as a Novel Member of the Mitochondrial RNA Helicase Network in *Trypanosoma brucei*

Yingzhe Mark Xia[1], Takuma Suematsu[1], Andres Vacas[1], Inna Afasizheva[1]

[1] *Boston University*

P54. Reconstitution and interaction mapping of RNA editing catalytic complexes in *Trypanosoma brucei*

Dmitrii Afonin[1], Clinton Yu[2], Takuma Suematsu[1], Andres Vacas[1], Lan Huang[2], Inna Afasizheva[1], Ruslan Afasizhev[1]

[1] *Boston University*

[2] *University of California*

P55. Substrate recognition in RNA editing may involve combinatorial interactions between dsRBDs and ZnFs in *Trypanosoma brucei* development.

Lars O'Hara[1], Cody Goode[1], Tyler Rodshagen[2], Suzanne M McDermott[3], Jorge Cruz-Reyes[1]

[1] *Department of Biochemistry and Biophysics, Texas A&M University*

[2] *Center for Global Infectious Disease Research, Seattle Children's Research Institute*

[3] *Center for Global Infectious Disease Research, Seattle Children's Research Institute. Department of Pediatrics, University of Washington School of Medicine*

P56. Networks of *Trypanosoma brucei* mitochondrial protein and RNA interactions revealed by CXMS and hiCLIP

Tyler Rodshagen[1], Cody Goode[2], Jorge Cruz-Reyes[2], Suzanne M McDermott[1]

[1] *Seattle Childrens Research Institute*

[2] *Texas A&M University*

P57. Disruption of a key regulatory editing complex profoundly affects cell growth, editing regulation, and editosome-holoenzyme assembly during development

Sunil K Sharma[1], Cody Goode[1], Tyler Rodshagen[2], Joshua Meehan[1], Alaisdair Ivens[3], Nicholas J Savill[3], Achim Schnaufer[3], Suzanne M McDermott[2], Jorge Cruz-Reyes[1]

[1] *Texas A&M University*

[2] *Seattle Children's Research Institute*

[3] *University of Edinburgh*

P58. Structural basis of the RNA editing cascade in trypanosome mitochondria

Yun-Tao Liu[1], Andres Vacas[2], Jonathan Jih[1], Xiaojing Zhao[3], Clinton Yu[4], Jane K.J. Lee[1], Takuma Suematsu[2], Md Solayman[2], Hong Wang[2], Xiaorong Wang[4], Lan Huang[4], Liye Zhang[3], Inna Aphasizheva[2], Z. Hong Zhou[1], Ruslan Aphasizhev[2]

[1] *University of California, Los Angeles*

[2] *Boston University*

[3] *ShanghaiTech University*

[4] *University of California, Irvine*

P59. Functional characterization of the transmembrane protein 18 (TMP18) in *Leishmania mexicana*

Kate Sagoe[1], Andreu Saura[1], Alexandra Zakharova[1], Vyacheslav Yurchenko[1]

[1] *Ostrava University*

P60. Using proteomics to uncover clues for RDK1 function in *Trypanosoma brucei*

Ashutosh P Dubey, Parul Pandey, Dinh Bui, Chukwujekwu Aleke, Joseph Smith

P61. In situ cryo-ET reveals RP2-dependent organization of intraflagellar transport and early flagellum assembly in *Trypanosoma brucei*

Mingqi Zhao[1], Sophia R. Staggers[1], Stella Y. Sun[1]

[1] *University of Pittsburgh*

P62. Exploring the Role of Major Surface Proteases in *T. brucei* Infection

Sophie Marcus-Wade[1], Jane Dias[1], Sandra Trindade[1], Catarina Gadelha[2], Bill Wickstead[2], Luísa M. Figueiredo[1]

[1] *Gulbenkian Institute for Molecular Medicine*

[2] *University of Nottingham*

P63. Deciphering the role of B cells in Chronic Chagas Cardiomyopathy pathogenesis

Shweta Deshpande[1], Carolina Duque[1], Sergio Gómez[2], Julio Mantilla[3], Paula Baustista[2], Lyda Rojas[2], Luis Echeverria[2], Martha Diaz[3], Robert Gilman[1], Monica Mugnier[1]

[1] *Johns Hopkins University*

[2] *Fundación Cardiovascular de Colombia*

[3] *Universidad Industrial de Santander*

Poster Session B

Thursday, September 10, 2026 · 7:00PM – 9:00PM

P64. Mechanisms Promoting Antigenic Diversification in Extravascular *Trypanosoma brucei*

Xinrui Li[1], Lulu Singer[1], Olivia Tea[1], Jaclyn Smith[1], Monica Mugnier[1]

[1] *Johns Hopkins University*

P65. Capturing the Antibody-VSG Arms Race During *Trypanosoma brucei* Infection

Lulu M Singer[1], Jaime E So[2], Alexander K Beaver[2], Monica R Mugnier[1]

[1] *Johns Hopkins Bloomberg School of Public Health*

[2] *Johns Hopkins University School of Medicine*

P66. The identification of putative pan-nitroaromatic drug resistance genes in *Trypanosoma brucei*

Kendal Faussete[1], Penny Lee[1], Galadriel Hovel-Miner[1]

[1] *Stony Brook University*

P67. Inhibition of phosphatidylinositol 3-kinase with peptidomimetics as a therapeutic approach for trypanosomes

Mengjin Gao[1], Lissa Cruz-Saavedra[1], Mulate Zerihun[2], Mahesh Kumar[2], Hayelom Berhe[2], Nir Qvit[2], Igor Cestari[1]

[1] *Institute of Parasitology, McGill University*

[2] *Azrieli Faculty of Medicine, Bar-Ilan University*

P68. Specific metabolic changes in host cells are required to induce dormancy in *Trypanosoma cruzi*

Leonardo Loch[1], Talícia dos Santos Silva[2], Paula Rego Barros Bittencourt-Cunha[1], Michel Augusto Silva[1], Carolina Borsoi de Moraes[2], Sergio Albuquerque[2], Sergio Schenkman[1]

[1] *Universidade Federal de São Paulo*

[2] *Universidade de São Paulo*

P69. Exploration of Phosphatidylcholine Remodeling in *Leishmania major*

Noah Mueller[1], Kai Zhang[1]

[1] *Texas Tech University*

P70. Ubiquitin-Conjugating Enzymes in *Leishmania infantum*

Olivia Baqui Damário Gomes[1], Caroline Torres[2], Giovanna da Costa Mancin[3], Lucas Henrique de Mello Facione[3], Izaira Tincani Brandão[3], Munira Muhammad Abdel Baqui[3], Felipe Roberti Teixeira[4], Marcelo Damário Gomes[3]

[1] *Faculty of Medicine of Catanduva; Federal University of Sao Carlos*

[2] *School of Medicine of Ribeirao Preto; Federal University of Sao Carlos*

[3] *School of Medicine of Ribeirao Preto*

[4] *Federal University of Sao Carlos*

P71. REDOX Biosensors for Analysis of Drug Effects on Bloodstream Form *Trypanosoma brucei*

Luiz Lola Pereira[1], Nyhal Metidji[1], Penny Lee[1], Galadriel Hovel-Miner[1]

[1] *Stony Brook University*

P72. SHARK-mediated knockdown of *Trypanosoma cruzi* Polo-like kinase (PLK) and Flagellar Pocket-Associated Cytoskeletal Components

Madeline F Malfara, Caroline Keroack, Jacqueline Lopez, Sophie Marcus-Wade, Christopher de Graffenried

P73. Characterization of RDK2 in bloodstream form *Trypanosoma brucei*

Chukwujekwu O. Aleke[1], Ashutosh P. Dubey[1], Shereen Nasrullah[1], Joseph T. Smith Jr.[1]

[1] *University at Buffalo*

P74. Inducible, Genome-Scale Overexpression ORFeome Library for Genetic Screens in *Leishmania donovani*

Corinne M Fargo[1], Tiago Rodrigues Ferreira[2], Jose M Requena[3], Kalyssa Kleinschmit[1], Stephen M Beverley[4], Galadriel Hovel-Miner[5], Phillip A Yates[1]

[1] *Oregon Health & Science University*

[2] *National Institute of Allergy and Infectious Diseases, NIH*

[3] *Universidad Autonoma de Madrid*

[4] *Washington University School of Medicine*

[5] *George Washington University*

P75. Characterization of *Trypanosoma* ICP and CatL Recombinant Protein

Maria Carranza[1], Milany Bruno[2], Daniel Lopes[3], Bernardo Gonzalez-Baradat PhD[3], Jayne Raper[4]

[1] *Macaulay Honors College, Hunter College, City University of New York*

[2] *Yalow Scholars Program, Hunter College, City University of New York*

[3] *Department of Biological Sciences, Hunter College, City University of New York*

[4] *Biology PhD Program, The Graduate Center, City University of New York*

P76. Trypanosome ICP Expression Under Stress

Sophia V Rodrigues[1], Berdardo Baradat[1], Jayne Raper[1]

[1] *CUNY Hunter College*

Abstracts

1. Molecular and structural dissection of VEX2 rewrites eukaryotic rules of helicase regulation to drive monogenic VSG expression

Leon Walther[1], Hideharu Hashimoto[2], Erik W. Debler[2], Michele Tinti[3], David Horn[3], Jamie N. Blaza[1], Chris H. Hill[1], Joana R. C. Faria[1]

[1] *University of York*

[2] *Thomas Jefferson University*

[3] *University of Dundee*

African trypanosomes evade the host immune response by periodically switching their homogeneous coat of Variant Surface Glycoproteins (VSGs). Strict regulation ensures that only one VSG expression-site (VSG-ES) is active at a time within a specialised sub-nuclear compartment - the expression site body (ESB). The 224 kDa superfamily 1 helicase VSG-Exclusion-Protein-2 (VEX2) is essential for maintaining this monogenic expression; its depletion leads to simultaneous expression of multiple VSGs within a single cell. However, the molecular mechanism underlying VEX2 function has remained elusive. Here, we present its first structural and biochemical characterisation. Using cryo-electron microscopy, we determined the apo structure of VEX2 at 3.5 Å resolution. Intriguingly, the "prong" subdomain, essential for autoinhibitory N-terminal interactions in its yeast orthologue Sen1, is absent. The lack of N-terminus-helicase complex formation in the cryoEM analysis and in co-immunoprecipitation assays support the absence of a stable N-terminal–helicase domain interaction. Instead, we show that the divergent N-terminus interacts directly with VEX1, a key component bridging the active VSG-ES to the spliced leader (SL) RNA locus for increased RNA processing. In vitro biochemistry confirms VEX2 possesses robust RNA/DNA unwinding activity, while in vivo DRIP-seq identifies endogenous R-loops as its substrates. Finally, CRISPR/Cas9 saturation mutagenesis demonstrates that VEX2 helicase activity is strictly required for monogenic VSG expression. Together, our data suggest VEX2 has evolved as a constitutively active helicase. We propose that its spatial confinement to the ESB bypasses the need for autoinhibition, allowing VEX2 to meet the extreme transcriptional demands required for parasite immune evasion.

2. A fine scale single-cell roadmap of the cell cycle and metacyclogenesis in *Trypanosoma cruzi*

Caroline D Keroack[1], Eric D Salomaki[1], Sophie Marcus-Wade[1], Madeline F Malfara[1], Jacqueline Lopez[1], Erin M Kennedy[1], Christopher L de Graffenried[1]

[1] *Brown University*

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi,* remains a significant global health burden with millions of people at risk of infection. The parasite undergoes several morphological changes during its life cycle allowing it to adapt to different host environments. Despite advances in transcriptomics, much remains unknown about the molecular mediators underlying key processes in epimastigotes, the insect stage of the parasite. Single-cell RNA sequencing has been used to define stage-specific gene expression in *T. cruzi,* including a recently generated atlas of the major life cycle stages. However, these data have not resolved the epimastigote cell cycle to the phase level. Here, we present a high-depth scRNA-seq dataset that resolves both the cell cycle and spontaneous metacyclogenesis. To confidently distinguish cell cycle from metacyclic clusters, we employed two growth conditions: one that enriched for cells undergoing metacyclogenesis and a second that favors proliferation. We identified markers of each cell cycle phase and validated their temporal expression by immunofluorescence microscopy. We also identified candidates for each stage of metacyclogenesis and have validated a previously uncharacterized RNA-binding protein as a marker of the first intermediate stage. Additionally, we have used the Short Hammerhead Aptazyme-Regulated Knockdown (SHARK) system to perturb a putative ubiquitin-conjugating enzyme E2, identified as a top marker of G1 phase. When depleted, we observe a significant growth reduction and kinetoplast defects. Together, these findings establish the first phase-resolved single-cell transcriptomes for the epimastigote cell cycle and metacyclogenesis, paired with functional validation, providing a powerful resource for understanding parasite biology.

3. DAC4-dependent chromatin loops coordinate efficient transcription in *Leishmania*

Manoj Koirala[1], Hanna Campbell-Irwin[1], Lissa Cruz-Saaverdra[1], Suellen Maran[2], Nilmar Moretti[2], Igor Cestari[1]

[1] *McGill University*

[2] *Universidade Federal de São Paulo*

Leishmania spp., the causative agents of leishmaniasis, organize their genes into polycistronic units (PTUs) that contain arrays of coding genes. Gene transcription initiates at the beginning of each PTU by mechanisms that remain poorly understood. We performed chromatin conformational capture and DNA sequencing (Hi-C) in *Leishmania mexicana.* We found a size bias in the chromosome interactions. A three-dimensional (3D) genome model suggested that large and intermediate-sized chromosomes co-interact and are distributed at the nuclear periphery, whereas small chromosomes co-interact extensively and share internal territories. Although chromosomes lacked silent regions, they still segmented into distinct transcribed compartments, here termed A and A', with their boundaries correlating with interchromosomal contact sites. Notably, we identified over 250 chromatin loops that enclose PTUs or tRNA genes, with their boundaries near PTU transcription start and end sites. Multiple loops from the same or different chromosomes co-interacted via their boundaries. Knockout of the nuclear deacetylase 4 (DAC4) disrupted chromatin loops and site-specific interchromosomal contacts, and its re-expression restored them, indicating that loops and interchromosomal contacts are DAC4-dependent. Importantly, DAC4 knockout led to a genome-wide decrease in gene expression, indicating that loop assembly is required for efficient transcription. The data support a model in which loop boundaries group RNA polymerase II or III transcription start and termination sites, likely coordinating transcription initiation and polymerase recycling. It also shows that loops are DAC4-dependent, and given that *Leishmania* lacks the loop architectural CCCTC-binding factor, this suggests an evolutionarily distinct mechanism for chromatin loop assembly.

4. An in vitro system to dissect antigen diversification in *Trypanosoma brucei* reveals donor template fate and selection requirements

Jaclyn E Smith[1], Kevin J Wang[2], Erin M Kennedy[2], Monica R Mugnier[2]

[1] *University of Maryland Baltimore*

[2] *Johns Hopkins School of Public Health*

The extracellular pathogen *Trypanosoma brucei* faces constant pressure from the host immune system. Using antigenic variation, the strategy of altering an antigen, here the Variant Surface Glycoprotein (VSG), to evade the pre-existing immune response, parasites can continuously escape the host leading to chronic infection. *T. brucei* encode numerous VSGs yet most of these are fragments. During early stages of infection, parasite populations expose a majority of the intact VSG repertoire to the host, depleting this reservoir of novel antigens and leaving few distinct encoded antigens capable of extending infection. To combat this shortage, parasites must make new VSGs through mosaic formation, combining two or more VSG fragments to generate novel antigens. We recently demonstrated that a blunt double stranded break induces mosaic formation where a homologous donor VSG template is utilized for local repair. To further dissect this mechanism, we developed an in vitro system where Lister427 parasites were modified to express the VSG AnTat1.1 which they endogenously lack. Within the silent VSG repertoire of this strain, only one donor VSG is available for recombination. By inserting naturally absent donor VSG fragments into the genome, we observed 100 bp of donor sequence is sufficient for mosaic formation and that donor VSGs are unmodified after use as a template. We are currently using this modular system to determine if mosaic recombination is error prone and capable of introducing de novo point mutations for unrestricted modification of VSGs. This system is a powerful new tool for making mechanistic discoveries in antigen diversification.

5. *Trypanosoma brucei* RNase H2 uses a non-canonical PIP motif to interact with the PCNA sliding clamp

Manon Andreck[1], Hideharu Hashimoto[1], Erik W Debler[1]

[1] *Thomas Jefferson University*

RNase H2 is an essential ribonuclease in *Trypanosoma brucei,* genetic depletion of which leads to accumulating RNA-DNA hybrids, so called R-loop structures, at Variant Surface Glycoprotein (VSG) expression sites and in increased VSG switching. RNase H2 interacts with the eukaryotic DNA sliding clamp Proliferating Cell Nuclear Antigen (PCNA), which recruits proteins through PCNA-Interacting Peptide (PIP) motifs with the consensus sequence QxxΨxxΩΩ. However, a canonical PIP motif could not be identified in TbRNase H2. Using a combination of structure prediction, x-ray crystallographic analysis of a PCNA-RNase H2-PIP peptide complex, and isothermal titration calorimetry, we discovered a non-canonical 6-residue PIP motif in the *T. brucei* RNase H2 subunit A, QKLLSF, which shows a binding affinity similar to that between human RNase H2A and PCNA. Unlike canonical PIP motifs, which contain a 310-helical turn, the TbRNase H2 A PIP adopts an extended conformation. Consistent with these differences, TbPCNA can only accommodate the *T. brucei* but not the human RNase H2A PIP motif, suggesting distinct modes of PIP recognition. As the *T. brucei* RNase H2A PIP sequence is invariant among kinetoplastids, highlighting its significance beyond *T. brucei,* the distinct interaction between RNase H2 and PCNA in kinetoplastids may be exploited for drug development for parasitic diseases.

6. TbRAP1 binds to selected RNAP II transcription termination sites to block RNAP II from reading through RNAP III-transcribed genes

Marjia Afrin[1], Lovlesh Thakur[1], Farheen Naz[1], Prem Kushwaha[1], Amit Gaurav[1], Jillian Gady[1], Bibo Li[1]

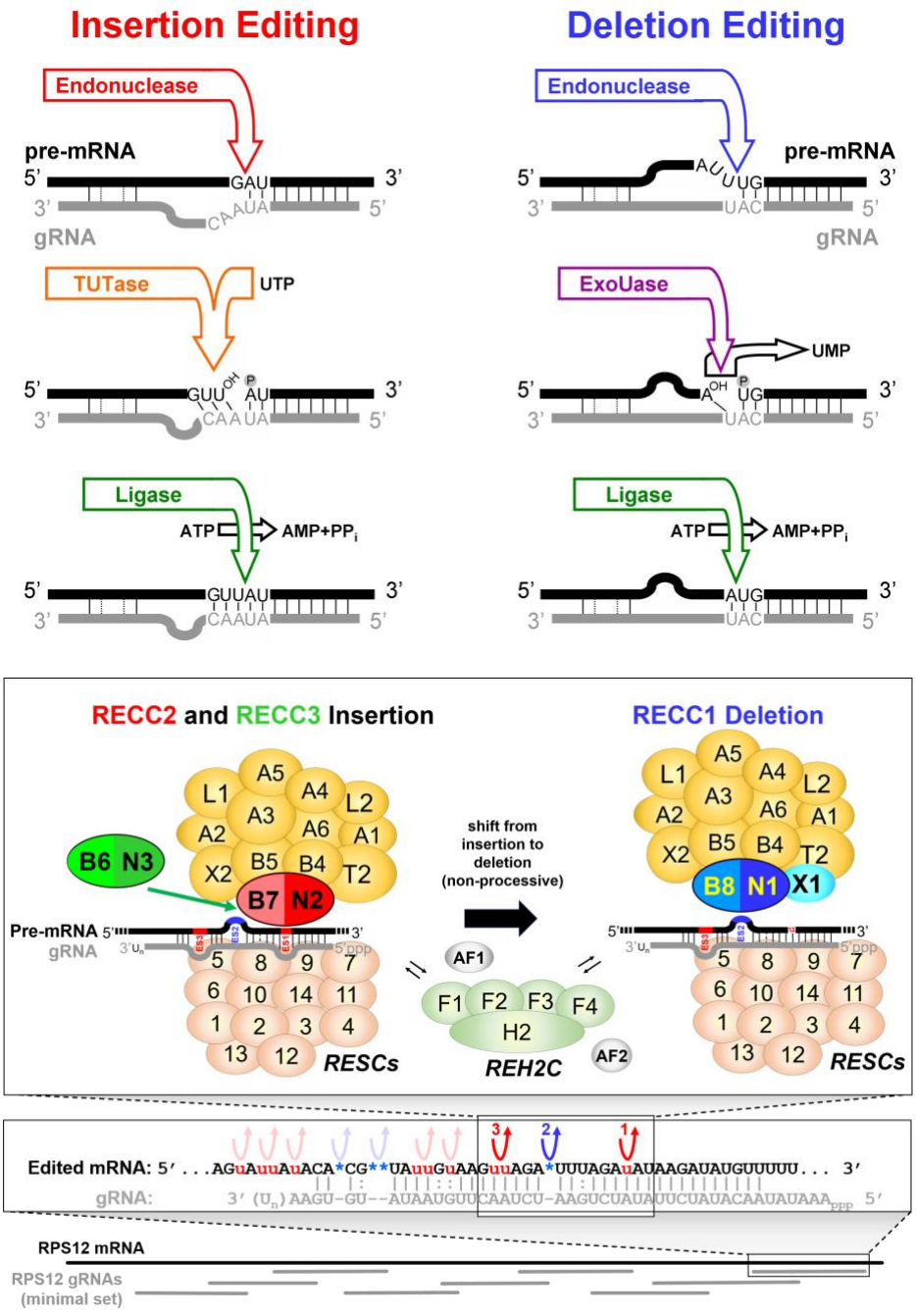
[1] *Cleveland State University*

Repressor Activator Protein 1 (RAP1) homologs are transcription factors that have both transcription activation and repression functions in gene expression regulation. They also associate with the telomere chromatin in eukaryotes from kinetoplastids to mammals. We have identified TbRAP1 as a key factor regulating VSG monoallelic expression in *Trypanosoma brucei* that causes human African trypanosomiasis and undergoes antigenic variation (VSG switching) to evade the host's immune response. We have demonstrated that TbRAP1's DNA binding activity is required for silencing all subtelomeric VSGs but one and that TbRAP1's binding to the active VSG RNA is essential for the active VSG's full-level expression. In the current study, our CUT&Tag-seq analysis showed that TbRAP1 and its interaction partner TbTRF bind multiple chromosome-internal loci, including a protein-coding gene on chromosome 9 (Tb86700) and multiple tRNA gene arrays positioned near transcription termination sites (TTSs) of RNAP II polycistronic transcription units (PTUs), but not individual tRNA genes within PTUs. In addition, we have found that TbRAP1 interacts with histone variant H3v, which is deposited at TTSs of RNAP II PTUs. TbRAP1 mutants defective in its DNA binding or TbTRF-interacting no longer binds to these loci, indicating both activities are important for TbRAP1's chromatin association profile. Importantly, RNA-seq, northern blotting, qRT-PCR analyses show significant upregulation of the TbRAP1-bound Tb86700 and RNAP III-transcribed tRNA and 5S rRNA genes in TbRAP1 conditional knockout cells, and depleting RNAP II subunit RPB9 suppresses the upregulation, strongly suggesting that WT TbRAP1 blocks RNAP II read-through activity across RNAP III-transcription regions.

7. RNA editing: An introduction

Jason Carnes[1]

[1] *Seattle Childrens Research Institute*



8. KREH3 helicase couples RNA processing and editing in trypanosome mitochondria

Takuma Suematsu[1], Xiaojing Zhao[2], Andres F. Vacas[1], Chenyu Fan[2], Clinton Yu[3], Md Solayman[1], Lan Huang[3], Liye Zhang[2], Ruslan Afasizhev[1], Inna Afasizheva[1]

[1] *Boston University*

[2] *ShanghaiTech University*

[3] *University of California, Irvine*

In the mitochondrion of the kinetoplastid protozoan *Trypanosoma brucei,* both minicircle and maxicircle genomes are transcribed into long primary RNAs that span multiple genes. The mitochondrial processome complex (MPsome) processively degrades minicircle transcripts in the 3'–5' direction until encountering duplexes formed by cryptic antisense RNAs. Resolution of these double-stranded intermediates generates mature ~50-nt guide RNAs (gRNAs), which direct U insertion/deletion mRNA editing. The MPsome also trims maxicircle-encoded mRNA precursors, with its exonuclease activity constrained by sequence-specific binding of the polyadenylation factor KPAF3. However, how gRNA–anti-gRNA hybrids are resolved and how processed gRNAs and pre-mRNAs are stabilized and directed into the editing pathway remain unclear. Here, we identify a DEAH-box RNA helicase, termed KREH3, as an ATP-dependent remodeling factor that binds gRNA sense–antisense hybrids, promotes MPsome arrest, and stabilizes mature gRNAs. KREH3 also coats properly sized pre-mRNAs, protecting them from degradation. We further show that KREH3 and the MPsome localize to antipodal foci flanking the kinetoplast DNA nucleoid, whereas mRNA 5' modification, 3' polyadenylation, editing, and translation occur in the mitochondrial matrix. Finally, we demonstrate the formation of "handover" assemblies between KREH3 and gRNA- and mRNA-binding factors. Together, these findings establish KREH3 as a mechanistic link between spatially segregated nucleolytic processing of primary transcripts and downstream RNA maturation and editing pathways.

9. Tandem zinc-fingers of an essential RNA helicase cofactor KH2F1 differentially impact editing of mitochondrial transcripts in *Trypanosoma brucei*

Suzanne M McDermott[1], Cody Goode[2], Tyler Rodshagen[1], Sunil K Sharma[2], Joshua Meehan[2], Alaistair Ivens[3], Nicholas J Savill[3], Achim Schnaufer[3], Jorge Cruz-Reyes[2]

[1] *Seattle Childrens Research Institute*

[2] *Texas A&M University*

[3] *University of Edinburgh*

Generation of functional mitochondrial mRNAs in trypanosomes involves guide RNA (gRNA)-directed mRNA editing, a process that is developmentally regulated between mammalian bloodstream and insect procyclic form *T. brucei,* in concert with metabolic changes. The mechanisms and factors underlying this regulation remained elusive; however, we recently showed that RNA helicase KREH2 mediates transcript- and stage-specific editing repression through mechanisms involving gRNA selection and/or utilization. Because RNA helicases act with cofactors that specify substrate choice and modulate activity, we examined KH2F1, a KREH2-associated protein in the REH2C editing complex. KH2F1, unlike typical RNA helicase cofactors, lacks G-patch or MIF4G domains and instead contains eight tandem zinc fingers (ZnFs). We assessed the importance of each ZnF via mutagenesis and exclusive expression in bloodstream form KH2F1-conditional null cells. Surprisingly, individually mutated ZnFs had markedly different effects on cell growth, REH2C association with other editing complexes, mRNAs and gRNAs, and on the abundances of fully edited mRNAs, including the developmentally regulated COX3 and ND8. Nucleotide-resolution RNA-seq further showed that the KH2F1 ZnFs had distinct effects on COX3 and ND8 editing and that each ZnF differentially impacted early editing fidelity via gRNA utilization. Together these data indicate that the KH2F1 ZnFs may direct specific mRNA-gRNA substrates to KREH2, including those whose editing differs between mammalian bloodstream form and insect procyclic form *T. brucei,* thereby likely playing a key role in developmental regulation of mitochondrial RNA editing. Correspondence: SM McDermott and J Cruz-Reyes

10. Extensive developmental regulation of COX3 Editing Is Driven by Reversible REH2C-Dependent ter-gRNAs in Trypanosome brucei

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Trypanosomatid protists offer several paradigms of eukaryotic biology, notably post-transcriptional U-indel RNA editing directed by guide RNAs (gRNAs), which remodels the mitochondrial (mt) mRNAome to enable translation. Several multiprotein assemblies are required, including the RNA Editing Helicase 2 Complex (REH2C). In *T. brucei,* this process is developmentally regulated in concert with the metabolic transition from glycolysis in bloodstream forms (BSF) and oxidative phosphorylation (OXPHOS) in insect procyclic forms (PCF). Maturation of mRNAs encoding components of mt respiratory complexes III and IV is therefore highly efficient in PCF but strongly silenced in BSF by unknown mechanisms. Here, we report an early editing checkpoint in cytochrome c oxidase subunit 3 (COX3) that is subject to extensive and reversible developmental control. In BSF parasites, COX3 editing repression at this checkpoint entails ~1000-fold REH2C-dependent inhibition of canonical gRNA-directed maturation together with activation of a "swarm" of gRNA terminators that aborts over 80% of early COX3 maturation. These REH2C-regulated terminators, detected in both PCF and BSF life cycle stages, define a new class of developmentally regulated inhibitory small RNAs, collectively termed ter-gRNAs. Strikingly, perturbation of REH2C proteins, particularly KH2F1, reversed most of the early COX3 repression and largely restored early maturation, indicating that the COX3 silencing is actively maintained and finely tuned by REH2C. Together, these findings, combining base-resolution RNA-seq, genetic manipulation, and transcriptomics, reveal a new layer of mitochondrial RNA regulation that involves crucial REH2C proteins, REH2C-regulatable ter-gRNAs, and a mechanistic model for reversible COX3 silencing during *T. brucei* development.

11. The KREPA3 L270 domain functions in gRNA selection and utilization during U Indel RNA editing in *Trypanosoma brucei*

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Trypanosoma brucei generates functional mitochondrial mRNA sequences by guide RNA (gRNA) directed U insertion and deletion RNA editing. Editing of some RNAs is extensive and differs between the cells' developmental stages. RNA editing occurs via multiple rounds of mRNA cleavage, U addition or removal and ligation. It is catalyzed by three similar RNA Editing Catalytic Complexes (RECCs) that differ in endo- and exo-nuclease content but have 12 proteins in common, most of which are essential. To illuminate the specific function of one of these RECC proteins, we analyzed the in vivo effects of an IDR-proximal L270R substitution in the KREPA3 protein. KREPA3 is critical for RECC integrity and is essential for editing in both developmental stages, but L270R only affects growth in bloodstream form cells. Exclusive expression of KREPA3 containing L270R substitution reduced the extent of editing of multiple mRNAs and changed the frequencies of editing at numerous sites. Expression of a mutant gamma ATP synthase allele (MGA), which circumvents the essentiality of editing, enhanced the effects of L270R by extending the phenotype duration in viable cells. Critically, the results show that the L270R substitution alters gRNA selection and utilization in editing, and indicates that RECCs function in gRNA/mRNA substrate recognition for editing. Furthermore, L270R reduces editing fidelity by greatly increasing the frequency of editing that contains base-pairing mismatches between mRNA and gRNA. Proximity to the IDR implies that the L270 domain functions via protein conformation changes that may also contribute to differential gRNA utilization between developmental stages.

12. Three-dimensional topology of kinetoplast DNA revealed by cryoET

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Kinetoplast DNA (kDNA) is a unique mitochondrial genome composed of thousands of minicircles and dozens of maxicircles that are topologically interlinked into a dense network often described as an Olympic-ring-like organization. Maxicircles encode mitochondrial rRNA- and protein-coding transcripts, many of which are cryptic and require guide RNA (gRNA)-mediated editing, whereas minicircles encode the gRNAs that direct this process. Although this catenated DNA network has been studied for decades, the three-dimensional arrangement of individual minicircles and maxicircles in the kinetoplast nucleoid has remained unresolved. Here, we apply IsoNet2-enhanced cryogenic electron tomography to investigate kDNA architecture in three dimensions. Our reconstructions reveal individual ring-like DNA densities consistent with minicircles, as well as larger DNA trajectories that may correspond to maxicircle-associated structures. Three-dimensional tracing of these densities enabled characterization of DNA packing and apparent interlinking relationships among individual circles. We further employed graph-based topology analysis, in which individual DNA circles are represented as nodes and apparent inter-circle catenations are represented as edges, enabling the construction of valency maps of the kDNA network. Together, our results provide a topological framework for understanding how the interlinked ring architecture of kDNA supports mitochondrial genome replication, segregation, and gene expression.

13. Cell cycle regulated kinetoplast proteins regulate kDNA replication, segregation and cell cycle progression in *Trypanosoma brucei*

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The kinetoplast is composed of two distinct classes of DNA molecules, larger maxicircles and smaller, more copious minicircles, which together form a catenated, chainmail-like network. Over 50 proteins are known to localise to the kinetoplast, with over 100 predicted to be involved with the replication and maintenance of the structure. Existing models of replication follow a localisation/function paradigm, with minicircle release and early replication occurring in the kinetoflagellar zone, and primer removal, gap filling and reattachment occurring at the S-phase exclusive antipodal sites. Here, we present a series kinetoplast proteins identified from a recent mitoproteome of *Trypanosoma brucei.* Using expansion microscopy, we demonstrate that these proteins show cell-cycle regulated sub-kinetoplast localisations. A novel repertoire of proteins are distributed at the kinetoplast basal face – opposite to the KFZ – which appear necessary for kinetoplast replication and segregation. Additionally, we have identified a pair of cytoskeletal-related proteins that localise consistently to the antipodal sites and are essential for parasite survival. RNAi of these proteins produces varying phenotypes, including minicircle loss, kDNA segregation failure and loss of viability. All proteins are taxonomically limited to parasitic kinetoplastids and therefore coincide with the emergence of a compact kDNA disc. Additionally, a cohort of these proteins appear to rely on an internal, hydrophobic targeting sequence and undergo mitochondrial import in the absence of a presequence.

14. Control of Minicircle replication by *Trypanosoma brucei*PIF1

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Faithful maintenance of the kinetoplast DNA (kDNA) network is indispensable for survival and life cycle completion of *Trypanosoma brucei.* Network replication involves a release, replication and reattachment mechanism for the thousands of minicircles and requires multiple paralogs of primases, helicases, ligases, and DNA polymerases with independently essential roles. Individual RNAi silencing of a subset of proteins involved in minicircle replication each produce multiply interlocked dimeric minicircles (fraction U) (Topo II, PIF1, POLIB, POLID), which may represent a replication stress signature. To better understand the division of labor during kDNA replication and its regulation, we leveraged our triple inducible system to study overexpression of wildtype PIF1. Cumate induced expression of Pif1 resulted in near homogenous expression of Pif1-FLAG in the mitochondrion with signal concentration near the kDNA after 24 hr. Prolonged cumate induction resulted in a loss of fitness after just 6 doublings and was accompanied by a pronounced increased in kDNA size. Additionally, PIF1 overexpression impacted cell cycle progression. Notably, PIF1-FLAG protein levels began to decline by day 4 of the induction, suggesting that PIF1 abundance may be tightly regulated. We are currently evaluating whether PIF1 overexpression leads to a selective increase in minicircle copy number. We also testing if minicircle replication is controlled by PIF1 abundance, which itself may be controlled by two known non-coding RNAs.

15. The dynamic intracellular world of *Trypanosoma cruzi*

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The obligate intracellular parasite *Trypanosoma cruzi* can infect most, if not all, nucleated mammalian cell types. Invading flagellated trypomastigotes differentiate into small ovoid-shaped amastigotes which divide by binary fission, often reaching >100 per cell. They then differentiate into non-replicative bloodstream form trypomastigotes. Following host cell lysis, trypomastigotes are released and propagate the infection, either by invading other host cells, or by being taken up in the bloodmeal of a triatomine insect vector. Several aspects of this intracellular life-cycle remain poorly described, limiting our understanding of parasite biology and disease pathogenesis. Key questions include: What is the role of the flagellum in invasion and egress, and is it required for dissemination within the host? What is the nature of the putative amastigote:trypomastigote differentiation intermediates observed both in vitro and in vivo? Do variations in the rate of intracellular replication impact on parasite persistence? In this presentation, I will describe recent work which shows that flagellar activity is necessary for parasite egress but not for invasion, and that the flagellum has a central role in transmigration, a process by which trypomastigotes can undergo multiple rounds of cellular invasion and egress, before establishing a productive infection. Furthermore, based on fluorescent imaging and EM studies, I will propose a new mechanistic pathway for the intracellular differentiation of amastigotes to trypomastigotes which involves passage through a flagellated 2N1K intermediate.

16. Unraveling the Role of Proteolytic Pathways in *Trypanosoma cruzi* Differentiation and Egress

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Trypanosoma cruzi, causative agent of Chagas disease, infects approximately 7 million people worldwide, yet the mechanisms underlying key intracellular steps remain incompletely understood. In mammalian cells, *T. cruzi* trypomastigotes invade, differentiate into replicative amastigotes, and then back into trypomastigotes, which egress to initiate new rounds of infection or transmission. Although essential for dissemination, egress regulation remains poorly understood. Previous studies suggested that parasite proteases contribute to late intracellular development, as protease inhibitors have been shown to inhibit parasite differentiation and egress. Using a dual-reporter parasites strain and ultrastructure expansion microscopy, we revisit the role of the cysteine protease inhibitor Z-FA-FMK. Our results show that Z-FA-FMK indirectly impacts egress by arresting parasite differentiation at an early stage. This arrest delays egress and leads to the release of immature forms, supporting a role for parasite proteases during late intracellular development. To identify additional egress regulators, we developed a semi-automated high-content imaging workflow for quantification of extracellular parasites. Screening 343 compounds enriched in protease inhibitors identified 14 non-cytotoxic hits inhibiting egress, of which six were selected for further characterization. Notably, two active compounds target a four-subunit intramembrane protease complex in mammalian cells. *T. cruzi* encodes structurally similar proteins, raising the possibility that related proteolytic machinery operates in the parasite. However, other inhibitors of this complex did not affect egress, suggesting a distinct or parasite-specific mechanism. Ongoing work aims to characterize their targets and how they interfere with differentiation and egress. Together, our findings support proteolytic pathways in *T. cruzi* late intracellular development and establish a platform to identify molecular regulators of egress.

17. Elucidating the in situ Spatial Organization of Intraflagellar Transport (IFT) at the Flagellar Base in *Trypanosoma brucei*

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Trypanosoma brucei, a protozoan parasite, is the causative agent of trypanosomiasis in humans and Nagana in animals. Its flagellum, which is essential for motility, sensing, and pathogenesis, is assembled by intraflagellar transport (IFT) complexes that move bidirectionally along the axoneme. In *T. brucei,* IFT is restricted to a subset of microtubule doublets (MTDs) that avoid the paraflagellar rod (PFR), unlike in Chlamydomonas reinhardtii, where anterograde trains dock along the B-tubules of all nine MTDs. Previous high-resolution microscopy suggested that IFT restriction initiates at the PFR-axoneme association site, but how trains organize at the flagellar base remains unclear. Here, using cryo-FIB-milling and cryo-ET, we identified two stages of anterograde IFT trains within the transition zone: initial and complete landing. Anterograde trains associated with both A- and B-tubules at the base, unlike their B-tubule specificity reported in Chlamydomonas. Because FIB-milling rarely captured all nine MTDs, we engineered enucleated "zoids" suitable for direct cryo-ET imaging. Zoids revealed a maximum of five simultaneous initial landing trains with alternating docking sites, unlike the nine-fold occupancy in Chlamydomonas. This pattern was further supported by observations from Magnify Expansion Microscopy. Initial landing trains persisted after depletion of IFT46, indicating that IFT landing is regulated independently of complete train assembly. Our results provide the first in situ structural evidence for spatial restriction of IFT trains at the *T. brucei* transition zone and identified the base as a regulatory hub for IFT entry. More broadly, this work underscores evolutionary adaptations in IFT compared with other ciliated or flagellated eukaryotes.

18. A Flagellar Membrane cAMP-phosphodiesterase in *Leishmania mexicana* is Critical for Colonization of the Sand Fly

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While cAMP signaling is critically important for kinetoplastid parasites, its mode of action is still obscure, and many of its biological functions have yet to be unveiled. Notably, *L. mexicana* promastigotes express a cAMP-PDE that is tethered to the flagellar membrane, a major site for both production and degradation of cAMP. Knockout of this cAMP-PDE gene profoundly impairs infection of *Lutzomyia longipalpis* sand flies and leads to defective motility in those few parasites that survive. Social motility in vitro is also strongly disrupted in this knockout line. Knockouts are still virulent in BALB/c mice, but remarkably, overexpression of the cAMP-PDE results in avirulence in the mouse, emphasizing the importance of correct cAMP regulation for parasite biology. While computational predictions suggested a single C-terminal transmembrane segment tethering this protein to the flagellar membrane, this hydrophobic segment appears not to be in the membrane. In contrast, a single myristoylation, without palmitoylation, at the N-terminus of the protein tethers a large proportion of this protein to the promastigote flagellar membrane. The first 10 amino acids of this PDE are sufficient to target GFP to the flagellar membrane, indicating that flagellar membrane trafficking information is contained within this limited segment of the protein. In some parasites, there is an accumulation of the cAMP-PDE at the distal tip of the flagellum, similar to the localization for some adenylate cyclases in *L. mexicana.* These results suggest that the tip of the flagellum is one center for cAMP regulation, as seen in *Trypanosoma brucei* by other researchers.

19. FLAM6 is a cAMP-binding protein that controls flagellar length, host cell infection, and vector colonization in *Trypanosoma cruzi*

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Cyclic AMP (cAMP) is a universal second messenger that plays a key role in eukaryotic cell differentiation and stress response. However, this signaling pathway remains poorly understood in *Trypanosoma cruzi,* the etiological agent of Chagas disease, where protein kinase A (PKA) is cAMP unresponsive and other canonical effectors have not been identified. Flagellar Member 6 (FLAM6) is an axonemal protein localized to the flagellar proximal domain with two predicted cyclic nucleotide binding domains (CBDs). The total lysate of recombinant bacteria expressing a truncated version of FLAM6 containing both CBDs was used to conduct a cAMP binding assay. Our results indicate that FLAM6 is a cAMP-binding protein, suggesting a putative role as a cAMP effector in *T. cruzi.* Functional characterization of FLAM6 through gene knockout using CRISPR/Cas9-based genome editing revealed significant defects in parasite proliferation, metacyclogenesis, flagellar length, mammalian cell infection, and colonization of triatomine hindgut. To determine whether cAMP binding is required for FLAM6 function, a TcFLAM6R447A-AB cell line is being generated to assess whether the disruption of cAMP binding reproduces the FLAM6-KO phenotypes. This cell line will provide direct evidence of the role of FLAM6 in the cAMP signaling pathway. The FLAM6 interactome analysis identified enrichment of proteins involved in translation, aminoacyl-tRNA ligation, phosphorylation, and intraflagellar transport. Together, these findings support the role of FLAM6 as a cAMP effector in *T. cruzi.* Our characterization of the first cAMP effector in this parasite will improve our understanding of the signaling pathways that govern parasite development and disease transmission in trypanosomes.

20. *Trypanosoma cruzi* SPC-mediated endocytosis serves as a redundant, dynamically regulated mechanism for heme acquisition

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Trypanosoma cruzi, the causative agent of Chagas disease, relies on an endocytic apparatus known as the cytostome-cytopharynx complex (SPC) for bulk endocytosis. Surprisingly, the molecular machinery underlying the SPC is dispensable for normal parasite growth in vitro, making the evolutionary retention of this complex organelle a long-standing mystery. Here, we present evidence that the SPC operates within a redundant nutrient acquisition system essential for *T. cruzi*'s survival. Through a palmitoylation proteome screen, we identified ABCA10, a putative ATP-binding cassette transporter involved in importing heme, an essential cofactor *T. cruzi* cannot synthesize. ABCA10 knockdown (KD) triggers upregulation of SPC-mediated endocytosis. Strikingly, simultaneous ABCA10 depletion and endocytosis blockade causes a severe growth arrest, exposing a conditional survival dependency. To validate this redundant heme acquisition strategy, we utilized an endocytosis-null mutant (MyAP KO). These parasites exhibit enhanced sensitivity to heme starvation. Furthermore, ABCA10 KD within the MyAP KO background phenocopies the severe growth defect observed during chemical endocytosis inhibition. Investigating this dynamic, we discovered that extracellular free heme directly modulates SPC activity. Heme starvation rapidly upregulates endocytosis, whereas excess heme represses it. Crucially, extreme heme concentrations do not impact baseline growth, indicating a coordinated regulatory response rather than cellular toxicity. These findings explain why *T. cruzi* retains the SPC: it serves as a dynamically regulated, redundant mechanism ensuring uninterrupted access to heme via direct acquisition or host hemoprotein digestion. This study highlights the SPC as a metabolic safeguard, exposing a vulnerability at the intersection of nutrient transport and endocytic regulation.

21. Investigating the Role of TcCIF1 Throughout the Life Cycle of *Trypanosoma cruzi*

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Trypanosoma cruzi, the etiological agent of Chagas disease, undergoes a highly coordinated cell division process that requires the ordered duplication and segregation of key organelles. In addition to the conserved eukaryotic cytokinesis machinery, trypanosomatids rely on lineage-specific regulators. One such protein, CIF1 (cytokinesis initiation factor 1), is essential in *T. brucei* for posterior cell-end formation and cleavage furrow progression. We are investigating the role of CIF1 in *T. cruzi* using CRISPR/Cas9-based tools to generate knockout and endogenously tagged cell lines. Deletion of TcCIF1 markedly impaired epimastigote proliferation, cytokinesis, and differentiation into metacyclic trypomastigotes. Endogenous cMyc-tagged TcCIF1 localized to the proximal end of the flagellum, near the basal bodies and FAZ, and allowed us to monitor TcCIF1 expression across all major life-cycle stages. Using double-tagged cMycCIF1/cMycCIF2 cell lines, we detected an interaction between TcCIF1 and TcCIF2 by IFA and co-immunoprecipitation followed by western blot, which was further supported by mass spectrometry analysis of cMycCIF1-interacting proteins. In conclusion, we demonstrate that TcCIF1 is required for epimastigote cytokinesis in *T. cruzi* and interacts with TcCIF2, another predicted cytokinesis regulator. We also identify an important role for TcCIF1 in metacyclogenesis. Ongoing work is assessing the impact of TcCIF1 on parasite infectivity and intracellular proliferation in host cells.

22. Dynamics within the *Trypanosoma brucei* subpellicular microtubule array

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Microtubules provide structural organization to cells via their assembly into large, macromolecular structures such as the mitotic spindle and flagellum. The protist parasite *Trypanosoma brucei* relies on a highly crosslinked network of subpellicular microtubules underlying the plasma membrane, known as the SPA, to regulate cell morphogenesis. Visualization of the SPA has traditionally relied on electron microscopy, which can be limited by section thickness, cellular helicity, and z-resolution. Current methods have not allowed full reconstruction of SPAs, which has made it difficult to understand how the SPA is organized and duplicated. In collaboration with the Advanced Imaging Centre (AIC) at the HHMI Janelia Research Campus, we have imaged multiple whole *T. brucei* using FIB-SEM at a resolution of 4x4x4 nm voxels, producing the first whole-cell reconstructions with sufficient resolution to map entire SPAs. These models comprise over 9,000 images, making manual segmentation strategies impractical, thus we developed a novel deep learning algorithm to identify, model, and quantitate SPA microtubules. Our data shows microtubule nucleation and termination sites within the SPA, where and when new microtubules are added to the SPA, as well as microtubule dynamics within dividing cells for the first time, such as how the cleavage furrow ingresses between two specific microtubules and how long, stabilizing microtubules are inherited during cell division. Our algorithm has removed 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.

23. ATP Synthase as an architect of mitochondrial remodeling

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Over the past decades, *Trypanosoma brucei* has evolved from a neglected tropical pathogen into one of the best-established experimental models for studying fundamental aspects of eukaryotic cell biology. Its remarkable biology, together with the availability of powerful genetic and biochemical tools, has enabled discoveries extending well beyond parasitology. My research has focused on the extraordinary plasticity of the parasite's single mitochondrion. During its life cycle, *T. brucei* alternates between two environments with fundamentally different energy demands, requiring extensive remodeling of mitochondrial ultrastructure and metabolism. In the mammalian bloodstream, parasites rely almost exclusively on glycolysis, while their simplified mitochondrion hydrolyzes ATP to maintain membrane potential. In contrast, the insect-stage mitochondrion is a highly branched organelle with abundant discoidal cristae that efficiently generates ATP by oxidative phosphorylation. This natural developmental transition provides a unique opportunity to investigate how mitochondrial architecture, bioenergetics and cellular differentiation are coordinated. Using this developmental model, my laboratory investigates the molecular mechanisms underlying mitochondrial remodeling. I will summarize our work on the regulation of ATP synthase activity and remodeling of the respiratory chain, with particular emphasis on our recent discovery that ATP synthase dimerization is dispensable for the reduced bloodstream-form mitochondrion but becomes essential during differentiation. Loss of ATP synthase dimers prevents proper cristae maturation and abolishes the developmental acquisition of oxidative phosphorylation despite normal assembly of the respiratory chain. These findings identify ATP synthase dimers not only as ATP-producing nanomachines but also as key organizers of mitochondrial architecture, revealing how membrane organization enables mitochondrial maturation and bioenergetic function during cellular differentiation

24. Lineage-specific innovations expand mitochondrial proteomes in kinetoplastids

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Trypanosoma brucei and *Leishmania tarentolae* are model kinetoplastids whose mitochondria contain a unique DNA network (kDNA) and exhibit specialized features, including developmental remodeling and RNA editing. By integrating mass spectrometry–based protein correlation profiling, which identified ~1,554 mitochondrial proteins in each species, with published datasets, we assembled inventories of 1,666 proteins in *T. brucei* and 1,594 in *L. tarentolae,* representing the most comprehensive kinetoplastid mitoproteomes reported to date. This expanded coverage includes ~124 previously unrecognized mitochondrial proteins. Although phylogenetically distant, trypanosomes and leishmanias share highly conserved mitoproteomes, ~700 components of which lack detectable homologs in other eukaryotes, highlighting extensive mitochondrial innovation. Comparison of *T. brucei* insect and mammalian stages revealed similar compositions but globally reduced mitochondrial protein abundance in the latter, except for components of calcium homeostasis and the alternative oxidase pathway. We also detected ten kDNA-encoded proteins, including products of pan-edited transcripts. These inventories establish a framework for functional analysis of previously uncharacterized mitochondrial factors and illuminate the innovations that have shaped kinetoplastid mitochondrial evolution.

25. Conserved and divergent mitochondrial assemblies in kinetoplastid parasites

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The mitochondrial proteomes of *Leishmania tarentolae* and *Trypanosoma brucei* comprise approximately 1,700 proteins, most lacking homologs in higher eukaryotes. We combined complexome profiling with cryogenic electron microscopy to define conserved and lineage-specific macromolecular assemblies that mediate mitochondrial functions in these kinetoplastid protozoans. By comparing two evolutionarily diverged model kinetoplastids and developmental stages with distinct metabolic demands, we generated a systems-level view of mitochondrial complex organization across parasite states. Comparative analyses demonstrate differences in the abundance and composition of respiratory, metabolic, RNA-processing, and other complexes across species and developmental stages, refining and extending existing annotations. We also uncovered previously unappreciated higher-order assemblies and stage-dependent remodeling of mitochondrial machineries. Structures of respiratory complexes $CIII_2$, CIV_2, and CV from *L. tarentolae* identify ten previously unrecognized nuclear-encoded subunits and resolve five mitochondrially encoded proteins, including products of pan-edited mRNAs. These reconstructions reveal architectural innovations, including a vestigial mitochondrial processing peptidase embedded within $CIII_2$, a CIV_2 dimer stabilized by an extensive clade-restricted interface, and a CV dimer containing the mitochondrially encoded ATP6 subunit. Together, our results provide a comprehensive framework for comparative mitochondrial biology in kinetoplastids and illuminate how these parasites assemble specialized mitochondrial machineries.

26. Influence of oxidative stress on the metabolic remodeling of *Trypanosoma cruzi* during the initial stages of cellular infection

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Trypanosoma cruzi encounters dynamic nutritional and oxidative challenges throughout its life cycle, requiring rapid metabolic adaptation to ensure survival, differentiation, and successful infection. To investigate how environmental stresses shape parasite metabolism, we established an untargeted GC–MS metabolomics workflow and applied it to epimastigotes exposed to nutritional deprivation and oxidative stress. These analyses revealed distinct metabolic signatures associated with each condition. Nutritional stress promoted alterations in pathways related to inositol, sphingolipid, and glycerophospholipid metabolism, suggesting membrane remodeling events linked to differentiation. In contrast, oxidative stress induced pronounced changes in pentose phosphate, sulfur, cysteine, methionine, and nucleotide metabolism, consistent with the activation of antioxidant and redox-supporting pathways. These results confirm that oxidative stress is not merely a damaging condition but may function as an important regulator of metabolic reprogramming in *T. cruzi.* To further evaluate the temporal relationship between redox fluctuations, we prepared a roGFP2-based redox biosensor to monitor oxidative dynamics during parasite growth and infection. The obtained sensor parasite may enable the identification of pathways associated with parasite adaptation, intracellular establishment, and differentiation, providing new insights into how redox signaling contributes to the progression of infection and host–parasite interactions.
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27. VEuPathDB.org: bioinformatic tools for genomic-scale data exploration, analysis, integration & discovery

Omar S Harb[1], David S Roos[1], on behalf of the VEuPathDB team

[1] *University of Pennsylvania*

Biomedical research is increasingly driven by Big Data: genome sequences & population diversity, all manner of multi-Omics datasets, etc. How can we effectively collect, store, maintain and integrate this information to ensure FAIR (Findable, Accessible, Interoperable, Reusable) data access, advancing biological understanding and defining targets for further study in the lab, field & clinic? VEuPathDB provides a robust, scalable data-mining resource, recognized by both the Global Biodata Coalition and the European Life Sciences Infrastructure (Elixir) as a Core Biodata Resource "of fundamental importance to the wider biological and life sciences community." VEuPathDB integrates data and tools to enable:

- accessing & interpreting information on genes (including recent integration of AI tools to summarize transcriptomic data), gene models, annotation, genomes, diversity, comparative genomics, epigenetics, transcriptomes & proteomes, nucleic acid & protein motifs, protein structures, interactomes, subcellular localization, metabolomics, pathways, phenotypic characterization, orthology-based functional inference, etc
- strategies for integrating & interrogating diverse datasets in silico, and analyzing/sharing your results -assessing & improving the quality/accuracy of available annotation, capturing expert community knowledge for the benefit of others
- analyzing your own datasets, for integration/querying in the context of other data, and disseminating these results, in compliance with funder & publisher requirements

VEuPathDB staff will be available during the meeting to answer your questions and receive feedback and suggestions.

28. Phospholipases as Essential Mediators of Lipid Remodeling in *Leishmania*

Salma Waheed Sheikh[1], Somrita Basu[1], Neha Biyani[1], Kai Zhang[1]

[1] *Texas Tech University*

Leishmania parasites undergo extensive membrane lipid modifications as they transition from the promastigote stage in sandflies to the amastigote stage in mammalian cells. Both promastigotes and amastigotes can acquire lipids by de novo synthesis and scavenging but the identity of lipid remodeling machinery remains unknown. Phospholipases are key enzymes that catalyze hydrolysis of membrane lipids and contribute to nutrient acquisition, signaling and lipid remodeling. While *Leishmania* parasites possess orthologs of phospholipase C (PLC1, PLC2, PLC3) and Lands cycle enzymes (PLA2-B and LPCAT), their roles in phospholipid turnover are understudied. In *Leishmania major,* targeted gene deletions followed by PCR and whole-genome sequencing suggest that PLC1, PLC3, and PLA2-B are essential whereas PLC2 is dispensable. Localization studies revealed diverse subcellular distributions, with PLC1 enriched at the flagellar pocket, the primary site of endo- and exocytosis, PLC2 localized throughout the cytoplasm, and PLA2-B in the endoplasmic reticulum. Lipidomic analysis showed that PLC1 overexpression reduces PE abundance during the stationary phase, implicating the role of PLC1 in phospholipid homeostasis and membrane remodeling. Ongoing virulence studies and DiCre-based knockout attempts will reveal stage-specific functions of these enzymes. Together, these findings will define the critical roles of phospholipid remodeling in *Leishmania* survival and virulence.

29. A reversible mesocyclic-like developmental state of *Trypanosoma brucei* is regulated by tryptophan-dependent nutrient sensing

Eva Doleželová[1], Hana Pavlisková[2], Tomáš Skalický[1], Martin Moos[1], Vladimír Varga[2], Alena Zíková[1]

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During its development in the tsetse fly, *Trypanosoma brucei* undergoes a series of differentiation events that generate distinct proliferative and non-proliferative life cycle stages. While the characteristics of these stages are well described, the signals controlling their formation remain largely unknown. We found that procyclic *T. brucei* cultures entering stationary phase undergo a reversible G1 cell-cycle arrest accompanied by pronounced morphological remodeling. These cells closely resemble mesocyclic trypanosomes from the proventriculus. Comparative transcriptomic analysis revealed a strong positive correlation between stationary-phase parasites cultured in vitro and parasites isolated from infected tsetse midguts four weeks after infection, indicating that the arrested population may represent a mesocyclic-like developmental state. Importantly, this transition was fully reversible upon transfer to fresh medium. To investigate the mechanism underlying growth arrest, we assessed mitochondrial function and cellular energetics. Respiration, mitochondrial membrane potential, ATP/ADP levels and proteomic analyses demonstrated that stationary-phase cells remain metabolically active, indicating that growth arrest is not caused by energy depletion. Consistently, supplementation with conventional carbon and energy sources failed to restore proliferation. In contrast, metabolomic profiling identified tryptophan as a key regulator of cell-cycle re-entry. Tryptophan supplementation restored proliferation and increased metabolic flux through pyrimidine biosynthesis, suggesting that activation of nucleotide metabolism is required for cell-cycle re-entry. Together, our findings establish a simple in vitro model of the mesocyclic stage and identify tryptophan as a previously unrecognized regulator of developmental progression in *T. brucei.*

30. Visualization of Apolipoprotein L1 at the plasma membrane of African Trypanosome: primary role in cell death

Kayla Leiss[1], Arva Demaliaj[1], Sara Fresard[1], Jayne Raper[1]

[1] *Hunter College*

African trypanosomes are extracellular parasites that infect humans and cattle. Trypanosome Lytic Factor (TLF), a specialized high-density lipoprotein (HDL), provides immunity to some species of trypanosomes in humans and non-human primates. Apolipoprotein-L1 (APOL1) is the lytic component, but 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. Other models suggest APOL1 forms pores in the mitochondrial membrane. To evaluate these models, we investigated APOL1 localization. Upon channel formation at the plasma membrane, extracellular cations would influx. To test this, we used a variety of fluorescent indicators and flow cytometry on *T. b. brucei* expressing an inducible serum-resistance associated (SRA) protein. We observed sodium and calcium influx in response to purified TLF. We hypothesized that the influx of cations would depolarize the plasma membrane, and could trigger mitochondrial depolarization. Depletion of extracellular calcium prevented calcium influx, but had no effect on mitochondrial membrane depolarization. However, replacing extracellular sodium with tetramethylammonium (TMA+), which cannot pass through the APOL1 channel, prevented mitochondrial depolarization, indicating that the influx of sodium was key. We tagged APOL1 with SNAP to visualize APOL1 localization by microscopy. SNAP-APOL1 localizes to the periphery of the parasite upon co-staining with markers for the mitochondria, lysosome and plasma membrane. These results suggest that sodium influx through APOL1 at the plasma membrane causes mitochondrial membrane depolarization, and thus extracellular influx of sodium is the driver of trypanosome death.

31. Glycosomal compartmentalisation and peroxin-mediated resistance to Allopurinol in *Leishmania infantum*

Fereshteh Fani[1], Philippe Leprohon[1], Marc Ouellette[1]

[1] *Centre de recherche en infectiologie du CHU de Québec*

Allopurinol, a purine analogue initially developed as a xanthine oxidase inhibitor, has clinical applications in the treatment of canine leishmaniasis. In *Leishmania,* purine metabolism is compartmentalized within the glycosome, a peroxisome-related organelle in which allopurinol is activated through the activity of hypoxanthine-guanine phosphoribosyltransferase (HGPRT). To elucidate the mechanism of action of allopurinol and the molecular basis of resistance to this drug, we performed stepwise selection for allopurinol resistance in *Leishmania infantum* and sequenced the genomes of the resulting resistant mutants. Whole-genome sequencing revealed mutations in three peroxin genes (PEX1, PEX6, and PEX11) and in nucleoside transporter gene (NT3). We confirmed their role in resistance, first by transfecting episomes of wild-type copies of the four genes in relevant mutants, which led to resistance reversion. Second, we introduced by CRISPR-Cas9 genome editing the mutations of the four genes in independent wild type cells and the edited parasites were resistant to allopurinol. Growth curves analyses with allopurinol and allopurinol riboside suggested that resistance in certain PEX mutants could be associated with impaired drug activation. We therefore tested the subcellular localization of HGPRT by confocal microscopy using a GFP-HGPRT fusion protein. The GFP-HGPRT displayed a distinct punctated fluorescence pattern consistent with glycosomal localization, in wild type cells as well as in NT3 and PEX11 edited parasites. However, PEX1 and PEX6 edited parasites showed a more diffuse GFP-HGPRT fluorescence signal, suggesting partial mislocalization of HGPRT to the cytoplasm. Small defect in glycosomal proteins can lead to resistance to allopurinol, a chemotherapeutic mainstay against canine leishmaniasis.

32. Genome-Scale Inducible RNAi library Screens in *Leishmania braziliensis*

Corinne M Fargo[1], Sloane M Domico[1], Stephen M Beverley[2], Phillip A Yates[1]

[1] *Oregon Health & Science University*

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The availability of genome-scale Loss-of-Function genetic screening technologies for *Leishmania* species has been limited. While recent advances in systematic CRISPR-Cas9 knockout collections and base editing platforms have expanded the options, these platforms lack the versatility of the inducible RNAi libraries available for African Trypanosomes. Here we describe generation of the first genome-scale inducible RNAi library for genetic screens in *Leishmania braziliensis,* one of the few *Leishmania* species with the capacity for RNA interference. To avoid the leaky expression common to inducible promoter systems in *Leishmania* species, inducible RNAi expression is mediated by conditional site-specific recombination via a dimerizable split-Cre recombinase (DiCre). We developed a novel method, Strand Displacement Duplication, to generate barcoded, genome-scale, DiCre-inducible stem-loop RNAi libraries from random genomic DNA fragments. The resulting plasmid library encompasses at least 99% of the *L. braziliensis* genome, and CRISPR-Cas9-assisted transfection of the library yielded ~880,000 independent clones. Results validating the RNAi library for both Loss- and Gain-of Fitness screens will be discussed.

33. The *T. brucei* Inducible Expression Library: Method Updates & Identification of Novel Nitroaromatic Resistance Genes

Penny Lee[1], Kendal Faussete[1], Anushka Shome[1], Elaine Mei[1], Allan Li[1], Nicole Hooshiari[1], Franck Dumetz[1], Galadriel Hovel-Miner[1]

[1] *Stony Brook University*

Human African trypanosomiasis results in fatal host infections if left untreated. Most of the available drug treatments have high host toxicity, drug resistance challenges, and unknown mechanisms and targets. The *Trypanosoma brucei* Gain-of-Function (GoF) library is a forward genetic tool that has proven useful in survival-based screening for the identification of drug resistance genes. While initially successful, this approach suffered from low target (GoF) enrichment, poor clustering on the flow cell, and required high-cost custom sequencing. Here, we addressed these concerns by developing a GoF-specific pull-down method with bead immobilization. Following adapter ligation, a biotinylated GoF oligo is used to generate libraries for pull-down using streptavidin-coated magnetic beads. The bead-bound library serves as a template for subsequent rounds of NGS preparation. This method enables library generation from less abundant samples while maintaining the precise requirements for Illumina sequencing. The addition of the GoF pull-down permits standardization in accordance with Illumina platforms. The streamlined GoF NGS pull-down protocol was used to evaluate the GoF library integrity following freeze-thaw. Subsequent statistical analysis showed that the majority of the GoF genes remained unchanged after freeze-thaw when compared to the source material. This demonstrates the stability of the GoF library and the effectiveness of the pull-down method for library generation.

34. Disruption of the DOT1A-RNaseH2 Interaction for Anti-Parasitic Therapy

Anna Paulson[1], Hideharu Hashimoto[1], Manon Andreck[1], Victoria S. Frisbie[1], Janna Kiselar[2], Laura Hartleb[3], Christian J. Janzen[3], Erik W. Debler[1]

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Essential cell biology processes that are unique to kinetoplastida can potentially be exploited for therapy development against neglected tropical diseases such as Human African trypanosomiasis, Leishmaniasis and Chagas disease. The histone H3 lysine K76 di-methyltransferase DOT1A is an essential protein that controls replication in *T. brucei* and most likely other kinetoplastida. RNAi-mediated knock-down of DOT1A abolishes replication completely while overexpression of DOT1A causes re-initiation of replication before the cell has completed S phase, implicating DOT1A as a master regulator of replication initiation. We previously discovered that the ribonuclease H2 complex (RNase H2) interacts with DOT1 enzymes in *T. brucei.* Here, we show that the DOT1A-RNase H2 interaction is specific to kinetoplastida, occurs with sub-nanomolar binding affinity, and completely inhibits DOT1A methyltransferase activity. Using x-ray footprinting (XFP), we identify key interface residues, which when mutated abolish the interaction. Since the identified DOT1A key residues are adjacent in sequence, we aim to mimic this interaction by a short peptide and replicate the DOT1A overexpression phenotype to induce parasite death. Due to strict sequence conservation of the key interface residues among kinetoplastida, disruption of the DOT1A-RNaseH2 interaction may represent a novel strategy to treat diseases caused by these parasites.

35. A genome wide antigen-antibody screen identifies a yeast-based therapeutic vaccine candidate for Chagas disease

Mira Loock[1], Lissa Cruz-Saavedra[1], Valeria Bernal Araujo[1], Igor Cestari[1]

[1] *McGill University*

Chagas disease is caused by *Trypanosoma cruzi* infection and results in decades-long, chronic, debilitating, and lethal disease. There is no vaccine available, largely due to the lack of vaccine targets. We generated a *T. cruzi* genome-wide library for yeast surface display and screened it with antibodies from Chagas disease patients to identify antigen-antibody interactions. We identified hundreds of *T. cruzi* antigens that are immunogenic in humans and mapped their antibody-binding sites at nucleotide resolution. Hundreds of immunogens were conserved across strains, divergent from human proteins, and expressed across all *T. cruzi* infectious forms, revealing potential vaccine targets. We vaccinated mice with a 63-amino-acid-long highly immunogenic region using recombinant protein (rIR1) or yeast surface expression (yIR1). yIR1 surpassed rIR1, reducing parasitemia in blood and tissues in a prophylactic, acute-stage vaccination model, validating IR1 as an immunogen and yeast as a vaccine vehicle for Chagas disease. Notably, therapeutic vaccination of chronically infected mice with yIR1 cleared parasites from muscle tissues and was accompanied by the production of α-IR1 antibodies and an increased proportion of CD8+ T cells, indicating that a vaccine for treating chronic Chagas disease may be feasible. The antigen-antibody screen revealed hundreds of immunogens for vaccine, diagnostic, and biomarker discovery, and uncovered yIR1 as a therapeutic vaccine candidate for Chagas disease.

36. Genomic and transcriptomic profiling of intracellular amastigotes implicates membrane lipid remodeling in amphotericin B tolerance in *Leishmania* (Viannia) *braziliensis*

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Background and aims. Tolerance to amphotericin B (AmB) in *Leishmania* limits its use as a last-line treatment for complicated cutaneous leishmaniasis (CL) and for infections refractory to first-line therapy. We investigated intrinsic mechanisms of AmB tolerance in *Leishmania* (Viannia) *braziliensis* clinical isolates at the intracellular amastigote stage, the form relevant to human infection. Methods. Thirteen *L*. (V.) *braziliensis* isolates from CL patients who had failed first-line treatment and/or AmB were classified by in vitro AmB susceptibility in intracellular amastigotes [2 tolerant / 11 susceptible]. Whole-genome sequences were compared across isolates, and the intracellular amastigote transcriptome of the AmB-tolerant isolate was profiled with and without AmB exposure, using RNA recovered from purified intracellular amastigotes. Results. Genomic analysis revealed copy-number gains spanning genes for N-glycan biosynthesis and biopterin/folate transport and metabolism in the tolerant isolates only. Differentially expressed genes (DEGs) in one tolerant isolate were enriched for membrane lipid pathways — inositol phosphate metabolism (KEGG; MetaCyc superpathway of inositol phosphate compounds), ether lipid metabolism, and sphingolipid recycling and degradation — consistent with remodeling of the membrane lipid environment targeted by AmB. Only two DEGs overlapped regions of copy-number variation, indicating that most expression changes are not attributable to gene dosage. Conclusion. Intrinsic AmB tolerance in *L.* (V.) *braziliensis* amastigotes is associated with transcriptional remodeling of membrane lipid metabolism rather than with gene-dosage changes, implicating pathways not previously linked to AmB tolerance. The work underscores the need to characterize drug responses in field isolates and establishes a workflow for transcriptomic analysis of purified intracellular amastigotes.

37. Comparative genetic validation of *Trypanosoma cruzi* proline pathway enzymes as potential drug targets

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Trypanosoma cruzi, the causative agent of Chagas disease, infects an estimated 6-7 million people, yet treatment still relies on only two drugs, benznidazole and nifurtimox which are both limited by efficacy and tolerability. New targets are therefore urgently needed. Because proline metabolism underpins several essential processes in *T. cruzi,* we evaluated its enzymes as drug-target candidates, disrupting each step of the L-proline pathway (P5CS, P5CR, PRODH, P5CDH) by two complementary strategies: constitutive CRISPR/Cas9 editing (CL Brener) and SHARK conditional knockdown (Y strain). The first indication of essentiality emerged during line generation: CRISPR/Cas9 yielded only heterozygous clones for P5CS, PRODH, and P5CDH, with homozygous nulls recoverable solely for P5CR, indicating that complete loss of the other three enzymes is poorly tolerated. Conditional depletion reinforced this: knockdown of P5CS, PRODH, and P5CDH reduced epimastigote growth by 64.4%, 66.6%, and 73.5%, respectively. Strikingly, the corresponding heterozygous deletions instead grew faster than controls (+20.6%, +154.7%, +42.7%), while P5CR behaved differently in both systems (homozygous KO, −20.6%; knockdown, no significant change). The reversal between heterozygous deletion and conditional depletion is not likely to reflect gene dosage alone. We propose it arises from compensatory remodeling of proline metabolism that stable, selected heterozygotes establish over time but acute knockdown bypasses, exposing the underlying growth requirement. Together these data identify proline-pathway enzymes as modulators of *T. cruzi* proliferation and prioritize P5CS, PRODH, and P5CDH for further validation. Ongoing work on metacyclogenesis and host-cell infection will test their relevance to transmission and pathogenesis.

38. Role of protein kinases in paromomycin resistance in *Leishmania infantum*

Raouia Fakhfakh[1], Jaideep Mallick[1], Arijit Bhattacharya[1], Adriano Coelho[1], Philippe Leprohon[1], Marc Ouellette[1]

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Paromomycin (PMM) is used in the treatment of visceral leishmaniasis, but high post-treatment relapse rates limit its broader application and highlight the need for systematic monitoring of resistance. However, the molecular basis of PMM resistance in *Leishmania* remains poorly defined. To identify genetic markers of PMM resistance, we selected *Leishmania infantum* clonal lines resistant to aminoglycosides (nourseothricin, hygromycin, and PMM). Whole-genome sequencing was performed and compared with the aminoglycoside-susceptible wild-type (WT) parental strain. Genomic analyses focused on single nucleotide polymorphisms, insertions and deletions, and gene copy number variations. Recurrent mutations were identified in two protein kinase genes, encoded on chromosome 14 (PK14) and 33 (CDPK1) and in a gene encoding an RNA recognition motif (RRM)-containing protein. These variants co-segregated and were strongly associated with high-level PMM resistance. Functional validation using CRISPR-Cas9-mediated gene editing demonstrated that introduction of the identified insertion into the PK14 gene in WT cells resulted in a >200-fold increase in PMM EC_{50}. Notably, PK14-edited parasites also exhibited alterations in CDPK1 and RRM-containing genes, suggesting a functional interaction between these loci. Resistance was largely reversible: episomal expression of the WT PK14 reduced by 50-fold resistance to PMM. Episomal expression of CDPK1 also reduced resistance but to a lesser extent. Together, we identified novel genetic markers underlying PMM resistance in *Leishmania* and suggest potential epistatic interactions between two kinases and a RRM-containing protein. Ongoing studies focus on functional characterization of PK14.

39. ECLIPSE: An intervention programme to improve patient journey and reduce stigma for people with cutaneous leishmaniasis in Brazil, Ethiopia and Sri Lanka

Helen P Price (on behalf of the ECLIPSE team)[1]

[1] *School of Life Sciences, Keele University, Newcastle-under-Lyme, United Kingdom*

Cutaneous leishmaniasis (CL) affects some of the most marginalized and underserved communities globally. While new WHO initiatives are increasing attention on skin-related NTDs, effective control requires a better understanding of the barriers to healthcare access and the wider impacts of CL on individuals and communities. I will present findings from the five-year interdisciplinary ECLIPSE programme, which aimed to improve the CL patient journey and reduce stigma in underserved communities in Brazil, Ethiopia and Sri Lanka. Using qualitative, quantitative and creative approaches, we explored the impacts of CL, levels of disease awareness, and the barriers and facilitators to diagnosis and treatment. Community Advisory Groups and Communities of Practice ensured that research tools and interventions were tailored to local contexts and shaped by stakeholders throughout. Findings from more than 300 interviews and 3,000 surveys showed that understanding of CL causes and transmission was generally low. Misinformation contributed to fear and stigma, while barriers to healthcare included travel distances, lack of childcare, fear of treatment side-effects, lost income, and limited awareness among both communities and healthcare professionals. The burden of CL was particularly severe in Tigray, Ethiopia, where conflict had devastated healthcare systems and contributed to rising case numbers through population displacement and increased contact with rock hyrax, a reservoir host of *Leishmania aethiopica.* Awareness of CL was extremely low, primary healthcare workers often viewed the disease as untreatable, and traditional remedies were widely used. We found evidence of severe stigma of affected individuals, which was linked to local beliefs about CL. The ECLIPSE team addressed identified challenges through the co-production of locally appropriate interventions, including awareness campaigns, community films, theatre, animations, media programmes, podcasts and healthcare training. Engagement with policymakers also contributed to changes in service provision. In Tigray, collaboration with the Regional Health Bureau and Ayder Hospital led to one new inpatient and seven outpatient CL treatment centres, which have treated more than 2,000 people since November 2023.

40. A genome-wide yeast surface display screen to identify *Trypanosoma cruzi* proteins involved in host cardiomyocyte infection

Lissa Cruz-saavedra[1], Mira Loock[1], Igor Cestari[1]

[1] *Mcgill University*

Trypanosoma cruzi (Tc) infects ~10.5 million people worldwide, causing Chagas disease. About 30% of chronic stage patients develop Chagas cardiomyopathy. The parasite factors, as well as host receptors engaged in cardiac cell invasion, remain largely unknown. We found that Tc switches surface virulence proteins at each infection cycle. Tandem mass tag-labelling and mass spectrometry (TMT-MS) confirmed variation in parasite surface protein expression during cell infection. The data suggest that either invariant proteins or conserved sequences within variant proteins may contribute to heart tropism. To systematically identify Tc proteins involved in cardiomyocyte infection, we performed a parasite antigen-host binding screen using a Tc genome-wide yeast surface display (YSD) system. In this approach, Tc genome fragments were expressed in yeast for surface display via the Aga1p-Aga2p proteins. We performed five rounds of YSD binding to H9c2 cardiomyocytes by either directly incubating the YSD library with H9c2 or incubating the YSD library with H9c2 after its pretreatment with Tc trypomastigotes to stimulate host surface receptor expression, followed by DNA sequencing of enriched and non-enriched libraries. Our preliminary data identified 11 confident candidates, including three trans-sialidases, three mucin-associated surface proteins, and five non-variant hypothetical proteins. RNA-seq and proteomics confirmed their expression in infectious forms. Using a machine learning model and the complete H9c2 surface proteome, we investigated potential host receptors involved in interactions. We uncovered previously described host receptors and potentially new interacting candidates for biochemical and cell-based validation. These results will help elucidate mechanisms of parasite host tropism and pathogenesis.

41. Phenotypic and genomic adaptation to in vitro culture of a novel *Trypanosoma cruzi* strain isolated from a cardiomyopathy patient

Jill Hakim[1], Sneider Gutiérrez[1], Carolina Duque[1], Dharani Moorthy[1], Jaclyn Smith[2], David Serre[2], Robert Gilman[1], Monica Mugnier[1]

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Chagas disease is caused by *Trypanosoma cruzi,* a parasite with a highly adaptive genome that remains understudied especially in parasite strains infecting human patients. Previously, we found gene copies of surface antigens maintained in diverse clinical isolates that had been lost in strains adapted to lab culture. Characterizing the genomes of clinical isolates is critical to understand the role that the human host plays in the evolutionary adaptation of the parasite. Here we present a resolved whole genome of the newly isolated parasite strain from a cardiomyopathy patient living in Baltimore, originally from Honduras. Following isolation, we subjected the parasite to 300 days of in vitro evolution. We found that after 20 passages, parasites had significantly faster growth and lower rates of spontaneous metacyclogenesis in in vitro conditions compared to counterparts from passages one and ten. Supporting the phenotypic assessment, we sequenced the P1 and P10 parasite populations. We found numerous low frequency variants, none of which reached fixation, enriched in non-coding regions. We detected copy number changes in the P10 population. We are preparing P20 and P30 for sequencing to evaluate genomic changes that may be associated with phenotypic adaptation. We have additionally successfully isolated several other *T. cruzi* parasites from human patients: of these, we have typed two as TcV, and will assemble these genomes for additional genomic analysis. These results illustrate the genomic plasticity of *T. cruzi* parasites and may identify genomic features dispensable for growth in culture that are nonetheless maintained during human infection.

42. *Trypanosoma cruzi* Infection and its Extracellular Vesicles Induce Host Nuclear Remodeling and DNA Damage Responses

Raul Alexander Gonzales-Cordova[1], Lucas Henrique Melo Falcione[1], Mayla Gabriela Zanchetta Venancio[1], Camila Gachet-Castro[1], Berta Barnadas Carceller[2], Carmen Fernández Becerra[2], Munira Muhammad Abdel Baqui[1]

[1] *Ribeirao Preto Medical School, University of Sao Paulo*

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Trypanosoma cruzi, the etiological agent of Chagas Disease, invades many cell types affecting numerous host-signaling pathways. Despite extensive research, the impact of *T. cruzi* on host nuclear dynamics remains poorly understood. We demonstrated that *T. cruzi* infection modulates host nuclear functions by downregulating RNA polymerase II activity and RNA-processing factors, including hnRNPA1, hnRNPA2B1, and U2AF35, leading to impaired host transcription and RNA processing. Additionally, infection induces host DNA damage, evidenced by double-strand breaks (DSBs) and activation of the DNA damage response (DDR), marked by phosphorylation of H2AX, 53BP1, and ATM in epithelial LLC-MK2 cells. We observed a temporal activation of the DDR pathway, with a peak of DNA damage at 12 hours post-infection, followed by repair mechanisms primarily through the non-homologous end joining (NHEJ) pathway mediated by DNA-PK. To elucidate the communication and interaction pathways used by *T. cruzi* to cause DNA damage in host cells, we investigated the role of extracellular vesicles (EVs) secreted during the infection. Then, we exposed LLC-MK2 cells to EVs derived from *T. cruzi* trypomastigotes. EVs were internalized in a time- and dose-dependent manner triggered DNA fragmentation and activation of DDR proteins, including γH2AX, γATM, and γDNA-PK, without affecting cell cycle progression. Importantly, these findings demonstrated that parasite-derived EVs are sufficient to induce host DNA damage response in the absence of infection. These results reveal that *T. cruzi* can alter host nuclear function and genome integrity both directly and via secreted EVs, highlighting novel mechanisms of host-pathogen interaction and potential targets for therapeutic intervention.

43. Parasite-Mediated Host and Vector Modulations underpinning Livestock Trypanosomiasis Transmission

Simon Kiplimo Tawich[1], Merid Negash Getahun[1], Christopher W Weldon[2], Souleymane Diallo[1], John Ngiela[1], Antoine Barreaux[1], Steeven Belvinos[1], Chiro Rungua[1], Victor Ndunyu[1], Daniel Masiga[1]

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The success of trypanosome parasites transitioning between hosts and life stages depends on their ability to manipulate host metabolism, immunity, and vector behavior. Tsetse flies' host behavioral plasticity and sensory adaptations are frequently linked to pathogen-favoring behaviors, but the underlying mechanism remains unclear. This study elucidated host physiological modulations and the vector's behavioral alterations, making trypanosome-infected hosts more attractive as compared to their uninfected counterparts. Analysis of volatile organic compounds (VOCs) by Gas chromatography–Mass spectrometry (GC-MS) confirmed that host infection with *Trypanosoma congolense* induced ionone emission and enhanced production of phenolic compounds. To uncover the molecular basis driving these modulations, we applied transcriptomics, metabolomics, quantitative real-time polymerase chain reaction (RT-qPCR), and behavioral assays. Trypanosome infection modulates cellular signaling pathways across multiple sensory modalities, including olfaction, vision, taste, thermosensation, and immunity in *Glossina pallidipes,* potentially favoring parasite transmission. Given the detrimental effects of infective flies in nature, which can repeatedly transmit infections during each feeding cycle, our intervention aims to better understand vector-host-parasite interactions and design lures that target more trypanosome-infected tsetse flies in nature. Following our Agent-Based Model (ABM) using identified trypanosome-associated odors, we showed that these baits doubled the catches of trypanosome-infected *G. pallidipes,* translating to a 75% reduction in the number of infected flies and 40% reduction in trypanosome prevalence in cattle. The interventions from this work will reduce disease pressure and promote livestock production in tsetse fly-endemic areas, translating to improved livelihoods.

44. From One Protease to Two Functions: Genome Plasticity and Visceral Adaptation in *Leishmania donovani*

Debolina Manna[1], Sunandini Bhattacharya[1], Shounak Jana[1], Kristína Záhonová[2], Suvendu Nandi[1], Deneshraj Srinivasan[1], Nilanjan Pradhan[1], Nidhi Pandey[1], Debarati Biswas[1], Mahitosh Mandal[1], Gayatri Mukherjee[1], Vyacheslav Yurchenko[2], Budhaditya Mukherjee[1]

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Highly plastic genome of *Leishmania* is thought to underlie its remarkable ability to adapt to diverse host microenvironments, yet how this plasticity translates into dermal versus visceral persistence remains unclear. GP63, a zinc metalloprotease, is a well-established determinant of promastigote infectivity, particularly in cutaneous leishmaniasis. Despite this, its role in amastigote stage and in visceral disease caused by *Leishmania donovani* remains undefined. Here, using an integrated approach combining phylogenetics, CRISPR-mediated genome editing, and biochemical analyses, we demonstrate that GP63 paralogues in *L. donovani* have undergone independent evolutionary diversification to support visceral infection. While GP63-paralogues encoded on chromosome 10, previously implicated in cutaneous manifestation caused by *L. major* were found severely truncated and dispensable in *L. donovani,* two distinct GP63-paralogues encoded on chromosome 28 (GP63_28) and 31 (GP63_31) were found 'essential' to establish visceral infection. LdGP63_31 facilitates promastigote attachment to macrophages by promoting lipid raft engagement and complement inactivation, thereby enabling host entry and subsequent amastigote differentiation. LdGP63_28 is essential for intracellular amastigote survival by suppressing host inflammatory pyroptosis. Structural and enzymatic analyses revealed preferential host localization and substrate specificities, possibly resulting from distinct amino acid substitution within conserved motifs of these proteases, indicating independent evolutionary adaptations. Importantly, loss of the LdGP63_28 more severely impaired amastigote reinfection than loss of its counterpart LdGP63_31. Together, these findings reveal coordinated functional specialization of GP63-paralogues ensuring visceral adaptation.

45. GT15 is required for *Trypanosoma congolense* cytoadhesion

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Trypanosoma congolense is an extracellular parasite that causes nagana, a parasitic disease of livestock in sub-Saharan Africa. Nagana challenges agricultural development and animal welfare, leading to productivity losses estimated at USD 1.2 billion/year. Our previous findings showed that *T. congolense* cytoadhesion determines disease severity and parasite proliferation, suggesting that targeting this host-parasite interaction might mitigate disease. Yet, the molecular mediators of cytoadhesion remain unknown. Using in vivo direct RNAi fragment sequencing (DRiF-Seq), we identified 12 genes whose silencing reduced cytoadhesion in a competition context. Among these genes is GT15, which in *T. brucei* encodes for a Golgi-resident, UDP-dependent beta1-4-Galactosyl-transferase involved in the elongation of poly-LacNAc repeats on protein N-glycans. To evaluate GT15 role in cytoadhesion, we engineered a *T. congolense* GT15 knockout cell line and quantified its cytoadhesion ability under static and flow conditions. Genetic ablation of GT15 drastically reduced parasite cytoadhesion to bovine endothelial cells across multiple host species, whilst re-introducing it by ectopic expression restored cytoadhesion to parental line levels. Moreover, preliminary lectin blotting and mass spectrometry analyses suggest that although the overall degree of N-glycosylation remained unchanged upon GT15 deletion, the glycosylation of specific surface proteins appears to be altered. Our work provide evidence that the mechanism of *T. congolense* cytoadhesion is conserved across mammalian hosts and may dependent on the expression of specific surface glycan structures.

46. Resolving the Transcriptional Landscape of *Trypanosoma brucei* Adipose Tissue Forms at the Single-Cell Level

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Trypanosoma brucei exhibits remarkable developmental plasticity to adapt to distinct host environments. Upon colonizing adipose tissue, the parasite adopts a specialized form termed the Adipose Tissue Form (ATF) that differs markedly from the canonical Bloodstream Form (BSF). Previous studies have shown that ATFs grow more slowly, activate β-oxidation pathways (Trindade et al., 2016), and display substantial population heterogeneity. To investigate the molecular basis of these adaptations, single-cell RNA sequencing (scRNA-seq) was used to compare the transcriptomic landscapes of ATFs and BSFs. In the blood, we found the two expected subpopulations, comprising replicative slender forms and non-replicative stumpy forms (Briggs et al., 2021). In adipose tissue, besides these two life cycle forms, we found a novel transcriptional trajectory that leads to an adipose-tissue-specific cluster of parasites characterized by being mostly in G0 and showing higher expression of mitochondrial genes and nuclear SLRNA. We named this subpopulation oxATFs. Higher transcriptional activity in mitochondria may be associated with activation of oxidative phosphorylation and reduced metabolic activity previously shown by our lab (Trindade et al., 2022). Intriguingly, nanopore sequencing showed that within the oxATF's cluster, SLRNA transcripts harbor a polyA tail of 150 nt, suggesting these transcripts may be used for cellular functions besides trans-splicing. SLRNA-polyA tailed transcripts have been previously shown by the Michaeli lab to be involved in cell-to-cell communication and social motility (Eliaz et al., 2017). Functional studies are ongoing to validate this adipose tissue-specific subpopulation. These findings underscore the power of scRNA-seq in revealing parasite heterogeneity in vivo and provide insights into tissue-specific adaptations that could inform the risk of relapses in African trypanosomiasis.

P47. Investigating the antigenic diversity of *T. brucei* VSG mosaics to study the evolution of host-pathogen interactions

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The refinement of the "Red Queen" hypothesis to describe host-pathogen interactions posits that hosts and pathogens co-evolve to survive in each other's presence. Hosts develop defensive mechanisms to fight infection and prevent recurrence, while pathogens implement strategies to evade host immune surveillance and persist. The parasite *Trypanosoma brucei* (T. brucei) exemplifies this perpetual evolutionary arms race. Within the mammalian host, it avoids humoral immune clearance primarily through antigenic variation, achieved by periodic switching of its Variant Surface Glycoprotein (VSG) coat. In addition, *T. brucei* expands its antigenic repertoire by recombining gene segments from different VSGs to generate mosaic variants. Both strategies depend not only on the extensive VSG gene archive but also on the "immunofocusing" of the antibody response toward a single epitope per VSG variant. Accordingly, recent studies indicate that substituting short amino acid stretches at key structural positions is sufficient to generate mosaics with distinct antigenic profiles. However, how such minimal sequence changes shape the antibody response remains unclear. To address this, the proposed project aims to compare the immunoglobulin gene repertoires elicited by infection with trypanosomes expressing highly homologous VSG variants and to map their target epitopes.

P48. Chromatin Profiling Unraveled: Comparing ATAC-seq Profiles in Monomorphic versus Pleomorphic *Trypanosoma brucei*

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During bloodstream infection, pleomorphic *Trypanosoma brucei* rely on quorum sensing (QS) to regulate the density-dependent differentiation of proliferative 'slender' cells into cell cycle-arrested 'stumpy' forms, which are pre-adapted for transmission to the tsetse fly. In contrast, monomorphic subspecies *T. b. evansi* and *T. b. equiperdum* have lost the capacity to undergo stumpy differentiation and are transmitted independently of the tsetse fly, enabling their expansion outside the tsetse belt. As monomorphism is thought to have arisen multiple times during trypanosome evolution, understanding the molecular basis of monomorphism may provide insight into how new monomorphic lineages emerge and become fixed within populations. Recent work using laboratory-selected monomorphic *T. brucei* Antat1.1 90:13 lines suggests that the transition to monomorphism may arise through an initially reversible "proto-monomorph" state associated with altered expression of key QS pathway genes, with subsequent mutations locking in the phenotype (Oldrieve et al., 2024). However, the mechanisms underlying these early stages remain poorly understood. To investigate changes in chromatin accessibility during the transition towards monomorphism, we optimised and validated ATAC-seq for *T. brucei* and applied it to three laboratory-selected monomorphic lines. By comparing chromatin accessibility between pleomorphic and monomorphic parasites, we sought to identify changes associated with the loss of developmental competence. Our results suggest that monomorphism can arise through multiple routes. Furthermore, changes in chromatin accessibility at procyclin loci were accompanied by changes in gene expression and, potentially, EP protein expression, highlighting unexpected transmission-association developmental pathways that may be remodelled during the emergence of monomorphism.

P49. Novel elements of translation termination in trypanosomatids

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Accurate termination of translation is essential for efficient gene expression, yet it has not been studied in *Trypanosoma brucei.* In addition, *Blastocrithidia nonstop* is a closely related trypanosomatid that has a non-canonical genetic code with all three stop codons reassigned to encode amino acids. Only UAA can function either as a stop codon, or as a glutamate codon. Hence, it remains unclear how termination of translation is determined in this organism. We investigate translation termination in *T. brucei,* an established model organism, to define the core termination components, and to functionally assess their counterparts in *B. nonstop.* We focus on eukaryotic release factor 1 (eRF1), the protein that recognizes stop codons and triggers translation termination. Its knock-down was generated in *T. brucei* and proved that depletion of the protein is lethal, and causes defects in termination. Importantly, this phenotype cannot be rescued by the ectopic expression of the *B. nonstop* eRF1. Indeed, comparison of the two eRF1 homologs reveals substitutions in residues previously shown to be crucial for eRF1 function, as well as several novel candidates. We hypothesize that for efficient termination, BneRF1 may require protein partner(s) to complement its function. Hence, we performed co-IP proteomics, and currently we are exploring the top candidate and its potential role in translation termination. Despite its fundamental role, translation termination remains poorly understood in trypanosomatids, and its investigation may also provide valuable insights for human diseases associated with translational defects.

P50. Understanding Recombination Mechanisms Within Transcriptionally Silent Variant Surface Glycoproteins (VSGs) in *Trypanosoma brucei*

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Trypanosoma brucei is an extracellular protozoan parasite endemic to sub-Saharan Africa that can effectively evade the host immune response in perpetuity by diversifying its antigens – namely, its variant surface glycoprotein (VSG). While the *T. brucei* genome contains 2000-3000 VSG genes, 80-90% of these genes are VSG pseudogenes without a continuous open reading frame. To make use of this limited genomic repertoire, the parasite can combine gene segments from various fragments to create "mosaic" VSGs, though the exact mechanism by which this occurs is unclear. While previous work has shown that induction of double-stranded DNA breaks (DSBs) in expressed VSG genes can trigger mosaic recombination, the extent to which this process is dependent upon structural requirements conferred by expression has been underexplored. Indeed, employing a readout reliant upon RNA-seq, we wondered whether we have limited the scope of repair outcomes we might observe. To answer this, we created parasites with transcriptionally silent AnTat1.1 in the genome and induced dsDNA breaks in the gene. Subsequent assessment of AnTat1.1 revealed not only mosaic VSG recombination qualitatively identical to that observed in expressed VSGs, but a high frequency of microhomology-mediated end joining-like deletion events. With this work, we have not only developed a system that allows us to observe more completely the scope of repair outcomes that might occur following DSB induction in a VSG, but we now have evidence to support the idea that VSG diversification, including mosaic recombination, is not exclusive to successfully transcribed VSGs but can occur throughout the genome.

P51. Investigating TERRA functions in TbTRF-mediated telomere maintenance in *Trypanosoma brucei*

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The protozoan parasite *Trypanosoma brucei* causes human African trypanosomiasis and regularly switches its major surface antigen, variant surface glycoprotein (VSG), to evade the host immune response. VSGs are expressed from subtelomeric loci in a monoallelic fashion, and we have shown that telomere proteins play important roles in regulation of VSG-mediated antigenic variation. The active VSG gene is located immediately upstream of the telomeric repeats and transcribed by RNAP I. In addition, the telomeric repeats downstream of the active VSG are transcribed into the telomeric repeat-containing RNA (TERRA), a long non-coding RNA resulting from RNAP I read-through transcription. We previously showed that TbTRF, a duplex telomere DNA-binding protein suppresses VSG switching and is essential for proper maintenance of the telomere integrity. Specifically, we have demonstrated that depletion of TbTRF results in increased TERRA levels, more telomeric R-loops (TRLs), more telomeric DNA breaks, and more recombination-mediated VSG switching events. Overexpression of RNase H1 suppresses these phenotypes. However, it remains unclear whether regulation of TERRA is the primary mechanism by which TbTRF maintains telomere stability. To answer this question, we established an inducible TERRA depletion system in *T. brucei,* which enables us to investigate the significance of TERRA regulation in TbTRF-mediated telomere maintenance. We are currently characterizing the consequences of simultaneous TbTRF and TERRA depletion, including effects on TERRA and TRL levels, VSG switching, and telomere recombination. These studies will determine whether TbTRF maintains telomere integrity and stability primarily through suppression of TERRA or through additional TERRA-independent mechanisms.

P52. Poly(A) polymerases and the dynamic regulation of VSG mRNA in *Trypanosoma brucei*

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The exceptional stability of variant surface glycoprotein (VSG) mRNA is required to sustain the high level of VSG protein expression and thus directly connected to *Trypanosoma* brucei's immune evasion. In this project, we investigated the molecular mechanisms behind VSG mRNA stability. Using Oxford nanopore sequencing we discovered that VSG mRNA – exclusively - bears poly(A) tails that are twice as long as the average poly(A) tail in *T. brucei*. We hypothesize that this specificity is likely due to conserved sequences in the 3' untranslated region (UTR). Upon scrambling a conserved 16-mer motif in the 3'UTR, that was previously shown to have a role in VSG regulation, we observed tail shortening of VSG mRNAs to average poly(A) tail lengths. We then sought to identify the enzyme that confers the VSG-specific tail elongation. For this, we knocked down potential poly(A) polymerase candidates and identified ncPAP1, a yeast Trf4 orthologue, as promising candidate. An ncPAP1 Knockdown led to a loss in tail length heterogeneity, as well as shortening of the VSG poly(A) tail, along with a cell growth defect. In contrast, knockdowns of other polymerases such as the canonical poly(A) polymerase PAP2 or ncPAP2 caused either a global, mild poly(A) tail shortening or no effect. Currently, we are combining the ncPAP1 knockdown with VSG reporter constructs to examine the temporal dynamics of VSG poly(A) tail shortening. This work sheds more light onto the post-transcriptional gene regulatory mechanisms of *T. brucei* and contributes to the understanding of its immune evasion strategy.

P53. KREH4 Emerges as a Novel Member of the Mitochondrial RNA Helicase Network in *Trypanosoma brucei*

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Mitochondrial gene expression in the parasitic protozoan *Trypanosoma brucei* depends on extensive post-transcriptional regulation, including RNA editing. RNA helicases play central roles in mitochondrial RNA metabolism by remodeling RNA and ribonucleoprotein complexes that mediate RNA processing and editing. Several mitochondrial helicases, including KREH1, KREH2, and KREH3, have been implicated in these pathways, but the full complement of participating helicases remains unknown. Here, we characterize a previously unannotated mitochondrial protein, which we named kinetoplastid RNA editing helicase 4 (KREH4). Complexome profiling places KREH4 within a mitochondrial RNA helicase interaction hub and ~1-MDa ribonucleoprotein assemblies, suggesting a role in coordinated RNA remodeling and regulation. Localization studies demonstrated that KREH4 resides predominantly in the mitochondrial matrix, a compartment enriched in RNA editing proteins and post-editing machinery. In addition, KREH4 associates with components of the RNA editing substrate binding complex (RESC) in an RNA-dependent manner, linking this protein to pathways involved in mitochondrial RNA regulation. Functional analyses in procyclic life-cycle stage revealed that neither KREH4 depletion nor expression of catalytically inactive mutant caused an obvious growth defect under standard culture conditions, whereas overexpression of wild-type KREH4 reduced parasite viability, indicating that proper regulation of KREH4 activity is important for cellular fitness. Together, these findings identify KREH4 as a novel component of the mitochondrial RNA helicase network in *T. brucei.* The association of KREH4 with both a prominent helicase hub and RNA editing machinery expands the repertoire of known mitochondrial helicases and suggests that coordinated helicase activities play a central role in organizing RNA-processing pathways.

P54. Reconstitution and interaction mapping of RNA editing catalytic complexes in *Trypanosoma brucei*

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Mitochondrial U-insertion/deletion RNA editing is carried out by RNA editing catalytic complexes (RECCs). Although recent cryo-EM structures have revealed the overall architecture of RECC1 and RECC2, the molecular basis of gRNA-directed mRNA cleavage and the roles of flexible regions that recruit downstream editing enzymes remain incompletely understood. Here, we combined heterologous reconstitution, in vitro activity assays, and in vivo mutagenesis to investigate these processes in *Trypanosoma brucei.* Structure-guided co-expression of the four RNase III/RNase III-like proteins that form the RECC2 endonucleolytic core together with the ZnF1–ZnF2 region of KREPA1 yielded a recombinant complex that catalyzes specific gRNA-dependent mRNA cleavage at an insertion editing site. These results define a minimal assembly sufficient for the first step of RNA editing. In parallel, structure-guided truncation of RECC subunits followed by affinity purification and mass spectrometry identified regions responsible for tethering the KREX1 exonuclease and the KRET2 TUTase–KREL2 RNA ligase module to the catalytic core. Together, these studies extend the structural framework of RECCs by defining minimal machinery sufficient for substrate cleavage and mapping flexible interactions that coordinate editing reactions.

P55. Substrate recognition in RNA editing may involve combinatorial interactions between dsRBDs and ZnFs in *Trypanosoma brucei* development.

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Trypanosoma and *Leishmania* protists generate translatable mitochondrial mRNAs through post-transcriptional uridine (U) insertion and deletion directed by antisense guide RNAs (gRNAs). In *T. brucei,* RNA editing is developmentally regulated by KREH2, a DEAH-Box Superfamily 2 helicase related to human DHX9 and *Drosophila* MLE. These three helicases contain N-terminal tandem double-stranded RNA-binding domains (dsRBDs). DHX9 and MLE carry two dsRBDs, which extensive studies implicate in substrate recognition and autoregulation, whereas the larger KREH2 protein contains seven dsRBD motifs of unknown function. KREH2 associates with mRNA and protein cofactors in REH2C messenger ribonucleoprotein complexes (mRNPs). One such cofactor, KH2F1, contains eight zinc-finger motifs and is proposed to act as an adaptor for helicase-specific interactions and functions. Current models posit that mRNA-bound REH2C base-pairs with complementary gRNA-bound RNA editing substrate complexes (RESCs) to establish mRNA-gRNA substrate hybrids. RNA editing catalytic complexes (RECCs) then transiently engage these hybrids. Using structure-guided systematic mutagenesis and genetic complementation, we tested the hypothesis that dsRBDs and ZnFs cooperate to promote substrate recognition, REH2C mRNP assembly, and REH2C/RESC association in both mammalian bloodstream and insect procyclic forms of *T. brucei.* We performed site-directed mutagenesis to disrupt protein or RNA interactions across these two lifecycle stages. Our studies support a model in which specific dsRBD and ZnF motifs are essential for bloodstream and procyclic form cell growth. Furthermore, they may act combinatorially to facilitate REH2C stability, RNA substrate recognition, and assembly of the editing-competent holoenzyme, thereby contributing to developmental editing regulation.

P56. Networks of *Trypanosoma brucei* mitochondrial protein and RNA interactions revealed by CXMS and hiCLIP

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Kinetoplastid RNA metabolism involves post-transcriptional RNA editing that creates translatable mitochondrial mRNAs through uridine insertions and deletions directed by antisense guide RNAs (gRNAs). Several multiprotein assemblies are required, including RNA Editing Catalytic, Substrate, and Helicase 2 Complexes (RECC, RESC, REH2C) that catalyze editing and bind or remodel mRNAs and gRNAs. The system faces challenges that are not fully understood, notably how its numerous components coordinate to facilitate mRNA-gRNA hybridization and edit hundreds of sites using many gRNAs. In addition, editing is developmentally regulated between bloodstream and procyclic form *T. brucei,* correlating with changes in parasite metabolism. Importantly, our data indicate that *T. brucei* REH2C components, including RNA helicase KREH2 and cofactor KH2F1, regulate developmental editing by modulating gRNA utilization; however, the precise molecular mechanisms underlying REH2C-mediated gRNA control remain unknown. To better understand molecular interactions within and between *T. brucei* REH2C, other mitochondrial proteins and RNAs, we are using a combination of in vitro and mitochondrial protein-protein crosslinking and in vivo protein-RNA crosslinking, followed by REH2C isolation and mass spectrometry or RNA sequencing. Our observed crosslinks are consistent with known REH2C interactions, reveal new interactions, and are validated by genetic and biochemical studies. The results provide spatial constraints to create detailed models of REH2C and its interactions with other editing complexes and mitochondrial RNAs. Crucially, while our initial experiments have been carried out in procyclic form *T. brucei,* the data provide an important reference for understanding how mitochondrial protein and RNA interactions differ between parasite life-cycle stages.

P57. Disruption of a key regulatory editing complex profoundly affects cell growth, editing regulation, and editosome-holoenzyme assembly during development

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U-indel RNA editing in *Trypanosoma brucei* mRNAs is directed by anti-sense gRNAs and developmentally regulated in a process mediated by the REH2C complex, which contains RNA helicase KREH2 associated with mRNA and protein cofactors. Two stably bound cofactors, KH2F1 and KH2F2, have been implicated in this regulation; however, their specific roles are under investigation (see other abstracts by these authors). In current models, mRNA-gRNA hybrid substrates are assembled via REH2C base-pairing with the gRNA-bound RNA editing substrate complex (RESC), through complementarity between their respective mRNA and gRNA cargoes. RNA editing catalytic complexes RECCs may then engage in iterative editing during transient contacts with hybrid substrates. Here, we used cell lines enabling conditional expression of REH2C proteins to assess effects on cell growth, developmental regulation, and the interaction and remodeling of REH2C/RESC higher-order RNPs in bloodstream and procyclic form (BSF and PCF) *T. brucei.* Most perturbations reduced growth in both stages. By contrast, KH2F2 loss was lethal in BSF but not in PCF. This stage-specific essentiality is consistent with our finding that KH2F2 stabilizes REH2C differentially during development. Notably, in BSF studies, conditionally expressed KH2F1 mutant variants relieved developmental repression of cytochrome c oxidase subunit 3 editing, while exhibiting more moderate effects on other substrates. In contrast, KH2F1 depletion in BSF abolished all editing action and REH2C/RESC interactions. Our studies also indicate substantial RESC remodeling mediated by REH2C. These findings support a model in which REH2C components govern editosome-holoenzyme assembly, substrate specificity, and developmental regulation. *Correspondence: SM McDermott and J Cruz-Reyes

P58. Structural basis of the RNA editing cascade in trypanosome mitochondria

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The molecular mechanism of uridine insertion/deletion mRNA editing in trypanosome mitochondria has remained elusive because of the highly dynamic nature of the underlying multi-enzyme machinery. Here, we define the editosome as a supramolecular assembly formed by the RNA editing substrate-binding (RESC) and catalytic (RECC) complexes, and present cryogenic-electron microscopy structures of the ~1 MDa RECC1 and RECC2. Resembling dragonflies, with a head, thorax-like core, tail and wings, these ribonucleoproteins mediate the U-deletion and U-insertion cascades, respectively. In each RECC, a tetrameric core containing one active and three inactive RNase III domains captures the gRNA–mRNA duplex, while auxiliary zinc fingers distinguish deletion from insertion sites and position the substrate for mRNA cleavage (step 1). Three peripheral OB-fold heterotetramers are flexibly attached to the core, forming a spatially adaptable reaction chamber. The tail recruits the exonuclease and uridylyltransferase that remove or add uridines (step 2), whereas the wings, coordinated by an architectural tRNA, position RNA ligases to seal the edited mRNA (step 3). Together, these structures reveal how gRNA-directed substrate recognition, mRNA cleavage, uridine deletion and insertion, and ligation are integrated within a single macromolecular machine. This architecture defines the mechanism of information transfer in RNA editing.

P59. Functional characterization of the transmembrane protein 18 (TMP18) in *Leishmania mexicana*

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Parasites of the genus *Leishmania* cause leishmaniasis, affecting millions worldwide, yet many of their proteins remain functionally uncharacterized. Transmembrane protein 18 (TMP18) is a protein whose homologue was found in the endosymbiont-possessing Noymonas esmeraldas to duplicate and neo-functionalize, giving rise to the bacteria-associated NesmTMP18e that plays an essential role in the spatial organization and vertical inheritance of the endosymbiont. However, the role of "parental" and universally present across Trypanosomatidae TMP18 remains poorly understood. Here, we ablated the TMP18-encoding gene (and established the add-back line) in *L. mexicana* and validated them by whole-genome sequencing, conventional PCR, and RT-qPCR. Transcriptomic analysis showed a broad range of differentially expressed genes. Immunoprecipitation of LmxTMP18 coupled with mass spectrometry has documented a significant enrichment of transmembrane proteins, and gene ontology analysis confirmed overrepresentation of the integral membrane components. The phenotypic characterization revealed no significant growth defect but pronounced disruption of the cell morphology. The localization of LmxTMP18 was found to be perinuclear, establishing this protein as a functionally significant component of the nuclear membrane in *L. mexicana.*

P60. Using proteomics to uncover clues for RDK1 function in *Trypanosoma brucei*

Ashutosh P Dubey, Parul Pandey, Dinh Bui, Chukwujekwu Aleke, Joseph Smith

Repressor-of-differentiation kinase 1 (RDK1) is one of two kinases identified in bloodstream form (BSF) *Trypanosoma brucei* parasites that were found to repress premature and spontaneous differentiation into the insect procyclic form (PCF). However, the effect of RDK1 RNAi was previously limited to RNA-Seq analysis and the expression of a single surface coat protein, EP1 procyclin. Thus, there remains a significant gap in knowledge on the impact of RDK1 expression on the proteome and the phosphoproteome in BSF *T. brucei* parasites. Here, we employ a proteomics approach to define the protein proximitome of RDK1 in BSF and to determine the impact of loss of RDK1 expression on the BSF proteome and phosphoproteome to uncover clues about potential mechanisms for RDK1 function. We found the RDK1 proximitome suggests that the C-terminal kinase domain of RDK1 is near the cell membrane and flagellum and likely oriented towards the cytoplasm. Unexpectedly, the most enriched proteins in the RDK1 proximitome were mitochondrial proteins with the RDK1 kinase domain oriented towards the mitochondrial matrix. RDK1 depletion causes bloodstream form parasites to upregulate several mitochondrial proteins and glycosomal proteins, several of which are upregulated in procyclic form parasites. Surprisingly, the mitochondrial phosphoproteome is largely unaffected by RDK1 depletion, while RDK1-dependent phosphoregulation is restricted to the cell membrane and flagellar subdomains. Current studies are ongoing to validate RDK1 mitochondrial localization and to identify direct RDK1 targets to delineate how RDK1 regulates BSF- to-PCF differentiation through its kinase activity.

P61. In situ cryo-ET reveals RP2-dependent organization of intraflagellar transport and early flagellum assembly in *Trypanosoma brucei*

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Retinitis pigmentosa 2 (RP2) is a conserved ciliary-base protein linked to human ciliopathies and required for proper cilium and flagellum assembly. In *Trypanosoma brucei,* RP2 associates with transition fibers at the flagellum base, positioning it near the transition zone–IFT interface where early steps of flagellum biogenesis are initiated. Our recent in situ cryo-electron tomography (cryo-ET) studies showed that IFT trains assemble and dock at the *T. brucei* flagellar base in a spatially restricted and highly organized manner, suggesting that the transition zone and associated basal structures form a platform for IFT entry. However, how ciliopathy-associated proteins such as RP2 contribute to this basal IFT organization and early axoneme assembly remains unclear. To define the structural consequences of RP2 depletion, we used in situ cryo-ET of enucleated *T. brucei* cells, or "zoids," an established cryo-ET-compatible system that improves visualization of the flagellar base. This approach enabled direct analysis of the basal body, transition fibers, transition zone, proximal axoneme, paraflagellar rod, and IFT organization in near-native cellular conditions. Our results reveal that RP2 depletion alters IFT morphology at the flagellar base and is associated with recurrent defects in central pair biogenesis and paraflagellar rod organization. Together, these findings support a model in which RP2 helps coordinate basal IFT organization with early axonemal assembly, extending our understanding of transition zone–mediated control of flagellum biogenesis in *T. brucei.*

P62. Exploring the Role of Major Surface Proteases in *T. brucei* Infection

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Trypanosoma brucei is an extracellular protozoan parasite that causes devastating disease in humans and cattle in Sub-Saharan Africa. The disease is almost always fatal if left untreated. These disease-causing parasites can exit the vasculature and colonize the tissue space, especially the adipose tissue (AT), but the process by which they do this remains unknown. A surface-protein RNAi screen was previously performed to begin answering these unresolved questions and several genes arose as potential key components in AT entry and/or colonization. Among these was one gene of a 21-member family of major surface proteases (MSPs). We propose there are 5 subfamilies of MSP (A-E) as opposed to the previously identified 3 (A-C) where MSPs C, D, and E represent completely unique proteins while A and B have several members with significant shared homology. When examining previous RNA-seq datasets comparing fat and bloodstream expression of these MSPs, MSP-C and D are considerably more upregulated in the fat than the blood. In this project, I am seeking to characterize the roles of MSPs C and D in *T. brucei* infection utilizing RNAi and mouse models. Mice infected with parasites depleted of MSP-D showed lower parasitemia and survived the infection 2x times longer than mice infected with wild-type parasites. We are currently investigating whether this effect is mediated by the immune response or by the interaction of parasites with vasculature or AT. These preliminary results strongly suggest a role for MSP-D in virulence and potential roles for the MSP family in host-pathogen interaction.

P63. Deciphering the role of B cells in Chronic Chagas Cardiomyopathy pathogenesis

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Chronic Chagas cardiomyopathy (CCC) is an inflammatory dilated cardiomyopathy caused by *Trypanosoma cruzi* infection and a leading cause of heart failure-related death in endemic regions. 20–30% of infected individuals develop CCC years to decades after initial infection. Research on CCC immunopathology has focused almost entirely on T cells and macrophages, and B cells in the myocardium have received little attention. Prior work in our lab using bulk RNA sequencing, single-cell RNA sequencing, and spatial transcriptomics identified greater B cell transcript abundance in CCC hearts compared to non-Chagas dilated cardiomyopathy (NCDCM) hearts. We hypothesize that B cells accumulate in the myocardium of CCC patients and contribute to disease pathogenesis through mechanisms not shared with NCDCM. To test this, we are performing immunohistochemistry and immunofluorescence microscopy on formalin-fixed paraffin-embedded cardiac tissue sections from CCC and NCDCM patients, staining with the B cell markers CD20 and CD79a. Preliminary data confirm the presence of B cells in CCC cardiac tissue, with apparent spatial association between B cells and fibrotic, collagen-rich regions. Initial co-staining with the endothelial marker CD31 suggests these B cells may be predominantly extravascular, though replicate staining is needed to confirm this localization. Ongoing work includes quantifying B cell abundance across CCC and NCDCM sections using QuPath, confirming localization with three-dimensional confocal imaging, and characterizing B cell subtypes. If B cells are found to actively infiltrate the myocardium, this would point to a new immune population driving CCC pathology and open the door to B cell-targeted treatment approaches.

P64. Mechanisms Promoting Antigenic Diversification in Extravascular *Trypanosoma brucei*

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Trypanosoma brucei, the causative agent of African trypanosomiasis, evades host immunity through antigenic variation by periodically switching its expressed variant surface glycoprotein (VSG). While previous studies have largely focused on the molecular mechanisms underlying VSG switching in vitro and in bloodstream parasites, how antigenic variation is regulated across distinct host niches during infection remains unclear. Recent work from our lab demonstrated that tissue-resident *T. brucei* exhibit significantly greater VSG diversity than bloodstream parasites, suggesting that tissue microenvironments may promote antigenic variation. Since experimentally induced DNA double-strand breaks (DSBs) can trigger VSG switching in vitro, DNA damage represents a potential mechanism linking tissue microenvironments to enhanced antigenic diversification. Here, we are investigating whether tissue-specific microenvironments promote DNA DSB formation in *T. brucei,* thereby contributing to enhanced antigenic diversification in extravascular spaces. Reporter-expressing *T. brucei* infections and γ-H2A immunofluorescence are being used to compare DNA damage levels between bloodstream and tissue-resident parasites. Future spatial transcriptomic analyses will be used to identify microenvironmental factors associated with parasite DNA damage and antigenic diversification. Preliminary analyses have revealed that γ-H2A-positive parasites were infrequently detected in the bloodstream at the time point associated with increased VSG diversification, suggesting that DNA damage may differ across host compartments. Ongoing optimization of immunofluorescence analyses is improving parasite identification and reducing tissue autofluorescence, enabling quantitative evaluation of γ-H2A positivity in tissue-resident parasites.

P65. Capturing the Antibody-VSG Arms Race During *Trypanosoma brucei* Infection

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Trypanosoma brucei, an entirely extracellular parasite, is able to evade antibody-mediated clearance using a sophisticated mechanism of antigenic variation. The parasite is covered in a dense coat of a single expressed copy of the antigenically variable Variant Surface Glycoprotein (VSG). During infection, *T. brucei* can "switch" the expressed VSG by accessing a genomic repertoire of thousands of VSG encoding genes, allowing the parasite to escape antibodies targeting the previous VSG. While the VSG-antibody interface drives *T. brucei* infection dynamics, the complexity of this system has limited research into the anti-VSG response in vivo. With potentially hundreds of VSGs expressed during a single infection, the anti-VSG response is impossible to capture using standard low throughput techniques. Here, we combined high throughput assays to elucidate the in vivo dynamics of the VSG-antibody interface in *T. brucei* infection in mice. 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 derived peptides, we profiled the VSG-specific binding repertoires of anti-sera from individual mice over the course of infection. We used IgM and IgG specific IPs to compare isotype-specific responses within and across infections. With our paired VSG-seq and PhIP-seq approach, we identified specific peptide epitopes and mapped them to VSGs expressed during infection, allowing us to track responses to common, dominant VSGs. Our results provide insight into the isotype-specific kinetics and binding profiles of the in vivo anti-VSG response.

P66. The identification of putative pan-nitroaromatic drug resistance genes in *Trypanosoma brucei*

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Both African and American trypanosomiases are treated with nitroaromatic drugs including nifurtimox, benznidazole, and fexinidazole. Nitroaromatic drugs can be associated with host toxicity and emerging resistance. Identification of genes that promote nitroaromatic drug resistance can uncover unknown drug mechanisms of action, resistance pathways, and perhaps even novel drug targets to be used in improved therapeutic approaches. The *T. brucei* whole-ORFeome Gain-of-Function (GoF) Library is poised to fill these significant gaps in understanding. Our goal was to identify genes that, when overexpressed, result in pan-nitroaromatic drug resistance. GoF genetic screens were completed for all three anti-trypanosome nitroaromatic drugs, in addition to isolating gDNA for metagenomic analysis, parasites were isolated from the screens that harbor putative drug resistance genes. Here, surviving populations from the nifurtimox and benznidazole screens were single-cell cloned and treated with all three nitroaromatic drugs, independently, to identify clones that displayed pan-nitroaromatic drug resistance. The resulting *T. brucei* clones were evaluated using a GoF PCR platform to identify the library specific gene that they harbor by traditional Sanger sequencing. Thus far, 22 putative drug resistance genes have been identified. Their predicted functions include, but are not limited to, stress response, signaling, DNA metabolism, regulation, and trafficking. A subset of putative pan-nitrodrug resistance genes have been cloned for overexpression in *T. brucei* to confirm their effects on resistance using cell viability assays to measure shifts in EC50. Confirmation of nitroaromatic drug resistance and subsequent elucidation of gene functions will illuminate mechanisms of action and resistance to advance future therapies.

P67. Inhibition of phosphatidylinositol 3-kinase with peptidomimetics as a therapeutic approach for trypanosomes

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Trypanosoma brucei causes African trypanosomiasis. The parasite evades host immunity through periodic switching of its variant surface glycoprotein (VSG) coat. We showed that phosphatidylinositol (PI) signalling enzymes are involved in VSG expression and switching. This process depends on the synthesis of PI(3,4,5)P3, which controls VSG gene silencing, and disruption of this pathway is lethal for the parasites. We aimed to determine whether PI(3,4,5)P3 synthesis is essential for *T. brucei* survival and represents a drug target. Metabolomic analyses identified PI, PI phosphates (PI3P, PI4P, PI5P), PI(4,5)P2, and PI(3,4,5)P3 species with ~100-fold more PIs than PI(4,5)P2 and PI(3,4,5)P3 in bloodstream forms. Recombinant *T. brucei* phosphatidylinositol 3-kinase (PI3K) was expressed and purified from Escherichia coli. Enzymatic analysis showed that it primarily phosphorylates PI and PI(4,5)P2, generating PI3P and PI(3,4,5)P3, respectively. Enzyme kinetics revealed a KM of 96.6 µM and a Vmax of 0.78 µmol·min^{-1} for PI, whereas PI(4,5)P2 exhibited a KM of 216 µM and a Vmax of 0.44 µmol·min^{-1}, indicating PI3K preference for PI as a substrate. We designed peptidomimetics against PI3K and identified two peptidomimetics that inhibit enzyme activity. IPP-102 and IPP-103 inhibited recombinant PI3K activity as competitive (Ki 4.4 µM) and uncompetitive (Ki 3.2 µM) inhibitors. They also inhibited the growth of bloodstream-form parasites with IC50 values of 5 and 10 µM, respectively. PI3K overexpression increased resistance to both compounds. The data show that PI3K-dependent PI and PI(3,4,5)P3 synthesis is essential for *T. brucei* growth and thus a drug target. We will test inhibitors in mice to determine their efficacy in vivo.

P68. Specific metabolic changes in host cells are required to induce dormancy in *Trypanosoma cruzi*

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The discovery of dormancy in *Trypanosoma cruzi* has reshaped our understanding of Chagas disease, explaining infection persistence and the failure of treatment. The absence of biomarkers and the dependence on bioimaging techniques make studying this process challenging. We aimed to elucidate the mechanisms by which the intracellular microenvironment is related to this process. To identify non-dividing parasites, the thymidine analog 5-ethynyl-2-deoxyuridine (EdU) was utilized. Our results revealed that the proportion of non-dividing intracellular amastigotes is decreased by high glucose availability and by the addition of glycerol in the cell culture medium in different strains. We also found a reduction in the nucleus volume with decreased nucleolus labeling with mAb L1C6, indicating chromatin condensation and reduced protein synthesis. Non-replicating cells were not labeled by antibodies that recognize phosphorylated histone H2A after DNA double-strand breaks and poly-ADP-ribosylation, indicating that replication arrest did not derive from a DNA damage response. The arrest was not affected by replacing the phosphorylation site of the alpha subunit of the translation initiation factor 2 (eIF2-alpha), known to arrest protein synthesis in parasites under nutritional stress or in cells expressing *T. cruzi* cyclophilin 19 that induces ROS. These data reveal that dormancy in *T. cruzi* is modulated by a specific microenvironment that induces in some parasites a large decrease in replication, transcription, and protein synthesis. Understanding how the host metabolism causes these may help to outperform Chagas disease treatments.

P69. Exploration of Phosphatidylcholine Remodeling in *Leishmania major*

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The role of lipid remodeling in the protozoan parasite *Leishmania major* remains poorly understood. Disruption of de novo phosphatidylcholine (PC) synthesis is lethal in cultured promastigotes but not intracellular amastigotes, suggesting that amastigotes acquire and remodel host lipids. Preliminary lipidomic analyses showed that glycerophospholipid composition differs substantially between parasite life stages, indicating that intracellular lipid metabolism may be more complex than previously thought. These analyses have identified lipid species absent or rare in mammalian hosts, raising questions about how parasites generate essential membrane lipids despite limited de novo synthetic capacity. Genomic evidence suggests that *Leishmania* possess Lands cycle enzymes, including lysophosphatidylcholine acyltransferase (LPCAT) which converts LPC into phosphatidylcholine (PC). This work investigates the role of lipid remodeling in generating glycerophospholipid diversity and parasite survival. We hypothesize that *Leishmania* relies on LPCAT-mediated remodeling to convert host-derived LPC into PC required for proliferation. To test this hypothesis, we are characterizing glycerophospholipid species and examining the function and essentiality of a putative LPCAT in *L. major.* These studies will clarify the role of LPCAT-dependent remodeling in parasite adaptation and may identify vulnerabilities in lipid metabolism that could be exploited therapeutically

P70. Ubiquitin-Conjugating Enzymes in *Leishmania infantum*

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Introduction Visceral Leishmaniosis (VL) is a severe neglected tropical disease caused by *L. infantum* and *L. donovani.* Exploring the molecular mechanisms behind the ubiquitination process could provide important insights into the molecular mechanisms of VL. Objectives This study aims to characterize the *L. infantum* orthologue gene of the human E2 ubiquitin-conjugating enzyme UBC8. Methods Ubiquitin-related genes in *Homo sapiens* and *Leishmania* were identified via in silico analysis. The ubiquitin (UB)-E2 enzymatic function of LinfUbc8 was confirmed by in vitro ubiquitination assays. The intracellular distribution of the enzyme in *L. infantum* promastigotes was determined by confocal immunofluorescence. LinfUbc8 knockout line was generated using CRISPR-Cas9. Results The LinfUbc8 protein shares a high degree of identity with *H. sapiens* UBC8. LinfUbc8 contains conserved catalytic residues, including Cys-89 and the HPN motif. LinfUbc8 was cloned, expressed in *E. coli* and purified using affinity chromatography. The purification of His6LinfUbc8 was confirmed by SDS-PAGE, showing the expected 29.8 kDa molecular mass with a yielded of 25 mg/L. When E1, His6LinfUbc8, UB, and ATP were combined under non-reducing conditions, a protein band of approximately 40 kDa was observed, suggesting the transfer of UB between E1 and His6LinfUbc8 to form thioester-linked UB. Confocal microscopy demonstrated that the protein is distributed throughout the cytoplasm, confined to a distinct subcellular structure extended from a region near the flagellar pocket toward the center of the promastigote. Finally, attempts to generate LinfUbc8 null mutants via CRISPR-Cas9 were unsuccessful, suggesting that LinfUbc8 is an essential gene for parasite viability. Conclusion LinfUbc8 is a cytosolic E2 enzyme in *L. infantum* with structural and functional similarity to human UBC8. The inability to obtain viable null mutants suggests that LinfUbc8 is essential for parasite development, reinforcing the central role of ubiquitination in *Leishmania.*

P71. REDOX Biosensors for Analysis of Drug Effects on Bloodstream Form *Trypanosoma brucei*

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Clinically relevant anti-trypanosome drugs are predicted to interact with cellular redox either directly or indirectly. Redox biology in Trypanosomatids is managed via an alternative thiol carrier termed trypanothione (T(SH)2), as opposed to glutathione. The synthesis and utilization of T(SH)2 requires unique and potentially druggable enzymes including tryparedoxin (Tpx). Reducing equivalents from T(SH)2 are utilized via Tpx for management of ROS stress, among others. Many anti-trypanosomatid drugs are predicted to disrupt cellular redox in a manner that results in parasite death. However, this prediction has not been thoroughly interrogated and traditional methods relied on cell extracts from killed parasites. Genetically encoded biosensors enable live cell quantitative, dynamic and compartment specific (cytosol or mitochondria) analysis of redox balance. The roGFP family of biosensors report redox changes via the oxidation of two cystines in the beta-barrel of the GFP biosensor. A previous publication demonstrated that roGFP, hGrx-roGFP (human glutaredoxin linked), and Tpx-roGFP (tryparedoxin linked) can accurately report perturbations in cytosolic and mitochondrial thiol redox in procyclic form *T. brucei.* Here we have utilized these constructs in bloodstream form *T. brucei* to quantitatively measure the effects of drug treatments on living parasites for the first time. Proof of principle experiments and preliminary data will be presented.

P72. SHARK-mediated knockdown of *Trypanosoma cruzi* Polo-like kinase (PLK) and Flagellar Pocket-Associated Cytoskeletal Components

Madeline F Malfara, Caroline Keroack, Jacqueline Lopez, Sophie Marcus-Wade, Christopher de Graffenried

Successful parasitism is dependent on the ability to proliferate in various cellular environments while evading the host immune system. *Trypanosoma cruzi,* the causative agent of Chagas disease, balances these needs by compartmentalizing endo- and exocytosis into discrete organelles. Most endocytosis occurs at the cytostome cytopharynx complex (SPC), a tube-like structure that has evolved from a feeding organelle in free-living ancestors. *T. cruzi* also contains a flagellar pocket (FP), an invagination of the plasma membrane where the single flagellum exits the cell body, which in the related parasite *Trypanosoma brucei* serves as the sole site of endo- and exo-cytosis. Despite differences in membrane trafficking, the FP appears similarly constructed in both parasites. In *T. brucei,* the conserved Polo-like kinase (PLK) is required for FP segregation and inheritance by controlling replication of a set of FP-associated cytoskeletal structures that are essential for FP shape and function. Both PLK and FP-associated structures are conserved in *T. cruzi.* Using ultrastructure expansion microscopy, we have visualized *T. cruzi* FP duplication, revealing that this process occurs much later in cell division than in *T. brucei.* Recent advancements in *T. cruzi* genetic manipulation now allow for conditional depletion of essential proteins via the Short Hammerhead Aptazyme-Regulated Knockdown (SHARK). Using this system, we show that depletion of *T. cruzi* PLK leads to an increase in cells at the latest stages of cell division and cells with aberrant DNA content, as previously shown. Additionally, we are employing the SHARK system to deplete important components of the FP-associated cytoskeleton.

P73. Characterization of RDK2 in bloodstream form *Trypanosoma brucei*

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Repressor-of-differentiation kinase 2 (RDK2) is one of two kinases identified in bloodstream form (BSF) *Trypanosoma brucei* parasites that were found to repress premature and spontaneous differentiation into the insect procyclic form (PCF). However, there is limited knowledge on how RDK2 represses BSF-to-PCF differentiation and how it differs from the repression exerted by another regulatory kinase, RDK1. Our preliminary findings demonstrate that depletion of RDK2 by RNA interference for up to 4 days does not push BSF parasites to achieve complete differentiation into PCF, as the expression of procyclin in RDK2 RNAi parasites is substantially lower than fully differentiated PCF parasites. This suggests that RDK2 RNAi parasites are awaiting additional signals to fully differentiate. Furthermore, subcellular fractionation of slender BSF, stumpy BSF, and PCF parasites and Western blotting for endogenous RDK2 reveals that RDK2 is predominantly released into the cytosolic fraction of slender BSF and PCF parasites. However, a notable fraction of RDK2 is retained in the organellar/membrane fraction of stumpy BSF parasites, whereas the cytosolic marker EF1a is released almost completely to the cytosolic fraction in all stages. This suggests that RDK2 is dynamically shuttled into subcellular niches. Here, we begin the initial characterization of RDK2 protein interactions and the effect of RDK2 depletion on the global proteome and phosphoproteome to uncover clues of RDK2 function in BSF *T. brucei* parasites.

P74. Inducible, Genome-Scale Overexpression ORFeome Library for Genetic Screens in *Leishmania donovani*

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Genome-scale genetic screening platforms in *Leishmania* species have primarily relied on loss-of-function approaches, including CRISPR-Cas9 gene knockouts, base editing, and RNA interference. Gain-of-function strategies, such as gene overexpression, represent powerful complementary approaches that have yielded important biological insights across a wide range of experimental systems. We constructed the first inducible, genome-scale overexpression libraries for *Leishmania donovani* using a rapamycin inducible split-Cre recombinase (DiCre) expression system previously optimized in our lab. Individual open reading frames (ORFs) were PCR amplified and cloned into barcoded inducible overexpression vectors using the Gateway system. The resulting plasmid library encodes 7,918 ORFs, or approximately 94% of *L. donovani* protein coding genes, with each ORF associated with one or more unique DNA barcodes to facilitate barcode sequencing (Bar-seq). Cas9-mediated transfection of the ORFeome library into *L. donovani* promastigotes yielded ~480,000 independent clones, which represents ~60-fold redundancy for each ORF. A proof-of-principle screen for α-difluoromethylornithine (DFMO) resistance revealed both its known target, ornithine decarboxylase, and a polyamine transporter not previously implicated in DFMO resistance. Additionally, a screen for resistance to the antileishmanial drug miltefosine uncovered eleven novel hits, including two suspected zinc finger RNA binding proteins. Results of these and additional screens will be presented.

P75. Characterization of *Trypanosoma* ICP and CatL Recombinant Protein

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Parasites of the genus *Trypanosoma* are unicellular eukaryotic blood organisms that cause African trypanosomiasis, also known as sleeping sickness in humans and Nagana in cattle. The presence of the cation channel-forming protein Apolipoprotein-L1 (APOL1) in a subset of high-density lipoproteins, called Trypanosome Lytic Factors (TLF), provide humans and some higher order primates immunity to the subspecies *Trypanosoma brucei* brucei. Our research has shown the cysteine protease Cathepsin L in *T. brucei* (TbCatL) can degrade APOL1, which could make the parasite resistant to cation channel-mediated lysis. It is further hypothesized that the Inhibitor of Cysteine Proteases in *T. brucei* (TbICP) can interact with TbCatL, thereby inhibiting its ability to degrade APOL1. The purpose of this project is to characterize and analyse the interaction between the proteins ICP and CatL from *T. brucei* brucei and *T. vivax* (the main trypanosoma species causing animal trypanosomiasis), as well as their interactions with APOL1 in an artificial bilayer. CHO-S cells and BL21 competent *E. coli* were transfected with plasmids containing the histidine-epitope-tagged DNA sequences for TbCatL, TbICP, TvCatL, and TvICP. The proteins were purified using nickel-column affinity chromatography and analyzed with western blot and silver staining. The activity of the enzymes TbCatL and TvCatL with the inhibitors TbICP and TvICP were measured using in vitro enzymatic assays. Revealing subspecies specific inhibition but raising the conundrum of in vivo interactions of CatL located exclusively in the lysosome and ICP in the cytosol.

P76. Trypanosome ICP Expression Under Stress

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African trypanosomes cause trypanosomiasis, a wasting disease, in humans and animals. Of the three subspecies of *Trypanosoma brucei* (*T. b. rhodesiense*, *T. b. gambiense*, and *T. b. brucei*), humans and some non-human primates are immune to *T. b. brucei* due to trypanosome lytic factor (TLF), a high-density lipoprotein component of the innate immune system that contains ApoL1. Following endocytosis, apolipoprotein L1 (ApoL1) inserts into endosomal membranes, is recycled to the plasma membrane, forms a cation channel, and induces osmotic lysis of the parasite. *Trypanosoma* Cathepsin L (TbCatL) can degrade ApoL1 and is inhibited by the endogenous inhibitor of cathepsin L (ICP) in vitro; however, TbCatL-ICP colocalization has not been confirmed in the parasite, suggesting ICP may have additional functions. We hypothesized that ICP changes distribution and intracellular expression in response to environmental stress to promote parasite survival. Because no antibodies exist to detect ICP, ICP was HA-tagged in trypanosomes using CRISPR-Cas9. Parasites were incubated for varied times under conditions to simulate stress experienced during the life cycle: starvation and 42℃ (heat shock). Cytosolic content was collected following hypotonic lysis and centrifugation. Proteins were separated by electrophoresis and analyzed by Western blot using anti-HA antibodies. Proteins were also detected and visualized through immunofluorescence. Preliminary results showed that stress conditions alter the expression and intracellular localization of ICP in *T.b. brucei*. Future experiments will examine ICP expression following ApoL1 exposure over time to further investigate its potential role in parasite survival in the host.

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