MPM XXIX

2018

9-13 SEPT

molecular
parasitology
meeting

marine biological laboratory
woods hole, ma

2018 - 29th Annual Molecular Parasitology Meeting

Sunday, September 9

2:00-5:00p	Registration	Swope Center
5:00-6:30p	Dinner	Swope Dining Hall
7:00-7:15p	Welcome to MPM	Lillie Auditorium
7:15-9:20p	**Session I**	Lillie Auditorium
9:00-11:00p	Mixer	Swope Meigs Room

Monday, September 10

7:00-8:30a	Breakfast	Swope Dinning Hall
9:00-10:10a	**Session II**	Lillie Auditorium
10:10-10:30a	Coffee Break	
10:30-11.30a	**Session III**	Lillie Auditorium
11:30-1:00p	Lunch	Swope Dinning Hall
1:00-2:45p	**Session IV**	Lillie Auditorium
4:00-5:00p	Free Time/ **Power hour**	Lillie Auditorium
5:00p-7:00p	BBQ Dinner	Swope Dinning Hall
7:00-9:00p	**Poster Session: A**	Swope Center
8:00-10:00p	Mixer	Swope Meigs Room

Tuesday, September 11

7:00-8:30a	Breakfast	Swope Dinning Hall
9:00-11:35a	**Session V**	Lillie Auditorium
11:35-1:00p	Lunch	Swope Dinning Hall
1:00-1:50p	**Session VI**	Lillie Auditorium
1:50-2:20p	Coffee Break	
2:20-3:30	**Session VII**	Lillie Auditorium
3:30-5:00p	Free Time/**EupathDB**	Lillie Auditorium
5:00p-7:00p	Dinner	Swope Dinning Hall
7:00-9:00p	**Poster Session: B**	Swope Center
8:00-10:00p	Mixer	Swope Meigs Room

Wednesday, September 12

7:00-8:30a	Breakfast	Swope Dinning Hall
9:00-11:30a	**Session VIII**	Lillie Auditorium
11:30-1:00p	Lunch	Swope Dinning Hall
1:00-3:00p	Free Time	

3:00-5:00p	**Poster Session: C**	Swope Center
4:00-5:30p	Mixer	Swope Meigs Room
5:30p-7:00p	**Keynote Lecture**	Lillie Auditorium
7:30-9:00p	Lobster Banquet	Swope Dinning Hall
9:00-12:00a	Dance Party/Mixer	Captain Kidd

Thursday, September 13

7:00-8:30a	Breakfast	Swope Terrace
10:00a	Housing Checkout by 10:00a	
9:00-10:00a	**Session IX**	Lillie Auditorium
10:00-10:30a	Coffee Break	
10:30-11:30a	**Session X**	Lillie Auditorium
11:30a	**Passing the torch:**	
	Meet the organisers of MPM2019-2021	
11:30-1:00p	Lunch & Departure	Swope Dinning Hall

***Note: Please remove your Poster by Lunch time the day following your presentation.**

Science at the 2018 Molecular Parasitology Meeting

Code of Conduct

PRINCIPLES: Scientific meetings are important mechanisms for rapid advancement and dissemination of new ways of thinking, particularly for pressing public health challenges such as parasitology. Clarity and openness from meeting participants is necessary for useful community feedback, appreciation, and debate.

THE CODE: To meet the goals of this meeting, it is each presenter's responsibility and duty to make claims that are exact and transparent. These claims should be supported with as much specific documentation as possible, within the time frame of the presentations.

SPECIFIC EXAMPLES: Conclusive functional genetic studies should be accompanied by gene names and database references. Discovery of new inhibitors or drug candidates should be accompanied by structures and quantitative descriptions of potency and selectivity. New diagnostic approaches should be accompanied by actual names, structures, or signatures of key analytes, when known. It should be possible to evaluate the value of new experimental methods for manipulating cells or analyzing their content, based on the experimental details provided.

WHY A CODE? The organizers merely offer some reminders for scientific conduct based on some time-proven principles. The 2007 MPM involved passionate pleas from many for more openness in some basic research presentations. Others argued for the need to protect potentially important intellectual property (IP). This simple code was assembled in close consultation with key leaders in the molecular parasitology community.

INTELLECTUAL PROPERTY AND CHOICES: In an age of translational research, the community fully recognizes the need for IP protection. Important data, or an experimental approach, that is not ready for disclosure can be protected until it is time. Yet, one cannot have it both ways: Vague descriptions of critical data or method should not be the central part of one's premature published abstract or public presentation.

REWARDS AND PENALTIES: The MPM is organized and supported by practicing scientists who choose to engage. No individual has judicial powers. Abstracts and talks that lack specifics, and merely posture, may meet community-wide disappointment. In contrast, detailed presentations which offer new insights of general use have always been promoted and celebrated.

We hope an understanding and regular observance of this code of conduct will add to another exciting scientific meeting.

Jayne Raper, Kirk Deitsch & Markus Meissner

ACKNOWLEDGEMENTS:

The organizers appreciate and thank the following:

Site support at the MBL, Woods Hole, MA:

- Melina Hale and Neil Shubin, Interim Directors, Marine Biological Laboratory;
- Kerri Mills and Paul Anderson for Conference support;
- Cathy Ham, Suzanne Livingstone, Barbara Stackhouse and Nancy Hadway for Housing and the Front Office;
- Erin Hummetolgu and Sodexo Dining Services staff;
- Noreen McNamara, Crystal Santiago, Cathy Hannigan, Jessica Berrios and all of the staff in Housekeeping;
- Richard Boudreau of the IT/AV Department for Web booths and other support, and all the staff in the Information Technology Department.

Award sponsors:

- Travel Awards: Special thanks to all the participants who donated $10 and more.
- The American Society for Tropical Medicine and Hygiene and Burroughs Wellcome Fund facilitated travel and speaking slots at ASTMH meetings.
- Cash awards, open access waivers and rapid decisions: PLos Pathogens
  Elsevier

Enjoy the meeting!

Jayne Raper
Kirk Deitsch
Markus Meissner

In Memoriam

Ruth Nussenzweig, a leader in the worldwide fight against malaria, died earlier this month (April 1). She was 89 years old.

Nussenzweig, a professor emerita of microbiology and pathology at New York University's Langone Medical Center, was chair of the department of medical and molecular parasitology for nearly 20 years—the first woman to hold the position. She was elected to the Health and Medicine Division of the National Academies in 2006 and the National Academy of Sciences in 2013.

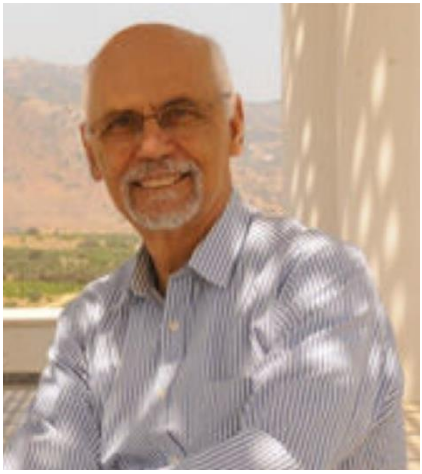
"Her work in the field of immunology serves as a guide for the development of a new generation of recombinant vaccines," Maurício Martins Rodrigues told *Agência FAPESP* in 2013.

Nussenzweig was born in 1928 to Jewish parents in Vienna. In 1939, her family fled to Brazil after Nazi Germany annexed Austria. When she was 18 years old, she enrolled in the medical school of the University of São Paulo, where she met Victor Nussenzweig, according to *Planeta Universeitario*. The two later married and collaborated together on immunology research for the next sixty years.

Over the course of her career, Nussenzweig dedicated herself to disease prevention and treatment. In 1967, she discovered that immunizing animals with irradiated *Plasmodium berghei* sporozoites protected the animals from the malaria-causing parasite. This research led to the development of the first human vaccine for malaria, which the World Health Organization recently approved for use in Africa (via *The New York Times*). She published more than 200 scientific papers in her lifetime, most recently coauthoring a study in *Scientific Reports* on vaccines against *Plasmodium vivax* in January of this year.

From:
https://www.the-scientist.com/the-nutshell/ruth-nussenzweig-malaria-researcher-dies-30042

In Memoriam



Fotis Kafatos. It is with a heavy heart that I share with you the sad news of the passing of Fotis Kafatos, our dear friend and former colleague.

Fotis trained with the distinguished entomologist and developmental biologist Carroll Williams in the Bio Labs and received his PhD in 1965. He stayed on as a faculty member in the Biology Department and quickly moved up through the ranks to Professor. Fotis later led discussions with colleagues in Biology that subsequently resulted in splitting of the Department into Organismic and Evolutionary Biology and Cellular and Developmental Biology (CDB). Fotis was the first CDB Chair. In 1994 CDB merged with Biochemistry and Molecular Biology to form the current MCB.

Fotis was influential on both sides of the Atlantic. Not only was he a gifted scientist and leader at Harvard, but he was also the Director General of the European Molecular Biology Laboratory and ultimately the President of the European Research Council.

He was a beloved scientist with a warm and engaging personality who will be greatly missed.

by Rich Losick

From:
https://www.mcb.harvard.edu/announcement/memoriam-fotis-kafatos/

Molecular Parasitology Keynote Lectures

2018: The Hidden Life of African Trypanosomes
Stephen L Hajduk, Department of Biochemistry & Molecular Biology
University of Georgia

2017: African trypanosomes and the art of being social
Isabel Roditi, Institute of Cell Biology, University of Bern, Switzerland

2016: Malaria sexual development: A challenging journey from the shadows into the spotlight
Robert Sinden Emeritus Proefssor, Imperial College, London UK

2015: A Physician-scientist's investigtion of amebiasis
Bill Petri University of Virginia, Charlottesville, VA, USA

2014: Molecular Malariology and Global Health: Discoveries, Predictions, Tests of Time
Thomas Wellems NIAID, NIH, Bethesda, MD, USA

2013: Navigating the trypanosome RNA jungle.
Elisabetta Ullu Yale School of Medicine, New Haven, CT, USA

2012: Pit bull or poodle? How are different breeds and developmental forms of Toxoplasma perfectly suited (or not...) to the challenge de jour.
John Boothroyd Stanford School of Medicine, Stanford, CA , USA

2011: Antigenic variation: it's all about persistence.
George Cross The Rockefeller University, New York, NY, USA

2010: The Major Challenges to Global Health in the Tropics & Beyond— from Insect Vectors of Malaria & of Other Parasitic or Viral Diseases
Fotis Kafatos Imperial College London, London, United Kingdom

2009: Understanding Parasites: A Foundation for their Elimination.
Ken Stuart Seattle Biomedical Research Institute, Seattle, WA, USA

2008: J, etcetera
Piet Borst The Netherlands Cancer Institute, Netherlands

2007: Rationale for the development of a malaria vaccine
Victor Nussensweig New York University, New York, NY, USA

2006: From genes to genomes: Insights into parasite biology
Dyann Wirth Harvard University, Cambridge, MA, USA

2005: How trypanosomes make fatty acids.
Paul Englund The Johns Hopkins University, Baltimore, MD, USA

2004: Should Today's Molecular Parasitologists Focus More on New Drug Discovery or Basic Research?
C. C. Wang University of California San Francisco, CA, USA

MPM Organizers 1990 – 2018

Molecular Parasitology Meeting XXIX, September 2018.
Meeting organizers: Markus Meissner, Jayne Raper, Kirk Deitsch

Molecular Parasitology Meeting XXVIII, September 2017.
Meeting organizers: Jayne Raper, Kirk Deitsch, Markus Meissner

Molecular Parasitology Meeting XXVII, September 2016.
Meeting organizers: Kirk Deitsch, Jayne Raper, Markus Meissner

Molecular Parasitology Meeting XXVI, September 2015.
Meeting organizers: Isabel Roditi, Upi Singh, Akhil Vaidya

Molecular Parasitology Meeting XXV, September 2014.
Meeting organizers: Upi Singh, Isabel Roditi, Akhil Vaidya

Molecular Parasitology Meeting XXIV, September 2013.
Meeting organizers: Akhil Vaidya, Upi Singh, Isabel Roditi

Molecular Parasitology Meeting XXIII, September 2012.
Meeting organizers: Artur Scherf, Kami Kim, Noreen Williams

Molecular Parasitology Meeting XXII, September 2011.
Meeting organizers: Noreen Williams, Kami Kim, Artur Scherf

Molecular Parasitology Meeting XXI, September 2010.
Meeting organizers: Kami Kim, Noreen Williams, Artur Scherf

Molecular Parasitology Meeting XX, September 2009.
Meeting organizers: Pradip Rathod, Dominique Soldati, Jay Bangs

Molecular Parasitology Meeting XIV, September 2008.
Meeting organizers: Dominique Soldati, Pradip Rathod, Jay Bangs

Molecular Parasitology Meeting XVIII, September 2007.
Meeting organizers: Jay Bangs, Dominique Soldati, Pradip Rathod

Molecular Parasitology Meeting XVII, September 2006.
Meeting organizers: David Sibley, Meg Phillips, Andy Waters

Molecular Parasitology Meeting XVI, September 2005.
Meeting organizers: David Sibley, Meg Phillips, Andy Waters

Molecular Parasitology Meeting XV, September 2004.
Meeting organizers: David Sibley, Meg Phillips, Andy Waters

Molecular Parasitology Meeting XIV, September 2003.
Meeting organizers: Steve Hajduk, David Roos, Debbie Smith

Molecular Parasitology Meeting XIII, September 2002.
Meeting organizers: Steve Hajduk, David Roos, Debbie Smith

Molecular Parasitology Meeting XII, September 2001
Meeting organizers: Steve Hajduk, David Roos, Debbie Smith

Molecular Parasitology Meeting XI, September 2000.
Meeting organizers: Christine Clayton, Kasturi Haldar, and Buddy Ullman

Molecular Parasitology Meeting X, September 1999.
Meeting organizers: Christine Clayton, Kasturi Haldar, and Buddy Ullman

Molecular Parasitology Meeting IX, September 1998.
Meeting organizers: Christine Clayton, Kasturi Haldar, and Buddy Ullman

Molecular Parasitology Meeting VIII, September 1997.
Meeting organizers: Daniel Goldberg, Marilyn Parsons, and Elisabetta Ullu

Molecular Parasitology Meeting VII, September 1996.
Meeting organizers: Daniel Goldberg, Marilyn Parsons, and Elisabetta Ullu

Molecular Parasitology Meeting VI, September 1995.
Meeting organizers: Daniel Goldberg, Marilyn Parsons, and Elisabetta Ullu

Molecular Parasitology Meeting V, September 1994.
Meeting organizers: Stephen Beverley, Paul Englund, and Barbara Sollner-Webb

Molecular Parasitology Meeting IV, September 1993.
Meeting organizers: Stephen Beverley, Paul Englund, and Barbara Sollner-Webb

Molecular Parasitology Meeting III, September 1992.
Meeting organizers: Jeffrey Ravetch, Lex Van der Ploeg, and Dyann Wirth

Molecular Parasitology Meeting II, September 1991.
Meeting organizers: Jeffrey Ravetch, Lex Van der Ploeg, and Dyann Wirth

Molecular Parasitology Meeting I, September 1990.
Meeting organizers: Jeffrey Ravetch, Lex Van der Ploeg, and Dyann Wirth

Travel Awardees

Blood donor variability as a modulatory factor in Plasmodium falciparum invasion phenotyping assays.
Thiam, Laty G.*; Aniweh, Yaw; kusi, Kwadwo A.; Niang, Makhtar; Gwira, Theresa M.; Awandare, Gordon A.*

Comparative assessment of PbSLTRiP (Sporozoite and Liver stage expressed Tryptophan Rich Protein) peptides as vaccine candidates against Plasmodium berghei in mice.
Quadiri, Afshana*; Singh, Agam Prasad*

Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi
Berná, Luisa*; Rodriguez, Matias; Chiribao, Maria Laura; Parodi-Talice, Adriana; Pita, Sebastián; Rijo, Gastón; Alvarez-Valin, Fernando; Robello, Carlos*

BioPalMar BEST TALKS – Registration Awards

Mind the traffic: a role for trafficking in the cell stress response and artemisinin resistance in P. falciparum
Henrici, Ryan*; Zoltner, Martin; Hart, Melissa; Edwards, Rachel; van Schalkwyk, Don; Baker, David; Moon, Rob; Odom John, Audrey; Field, Mark; Sutherland, Colin*

Impact of malaria-protective glycophorin polymorphism on Plasmodium falciparum invasion
Kariuki, Silvia N.*; Marin-Menendez, Alejandro; Leffler, Ellen; Band, Gavin; Rockett, Kirk; Macharia, Alex; Makale, Johnstone; Nyamu, Wilfred; Ndung'u, Francis; Kwiatkowski, Dominic; Williams, Thomas; Rayner, Julian*

Session I: Seeing is Believing

Chairs: Matthew Child and Veronica Jimenez

Sunday, September 9, 2018

7:00 pm – 9:00 pm

FT1: CryoEM Reveals Translocation Mechanism in Malaria Parasite Effector Export

***Ho, Chi-Min**; Beck, Josh R.; Lai, Mason; Cui, Yanxiang; Goldberg, Daniel E.; Egea, Pascal F.; Zhou, Z. Hong*

FT2: Flagellar cAMP signaling directs trypanosome navigation through the tsetse fly vector

***Millius, Sebastian**; DeMarco, Stephanie F.; Rehmann, Ruth; Wenzler, Tanja; Florini, Francesca; Roditi, Isabel; Hill, Kent L.*

FT3: Plasmodium falciparum displays heterogeneous binding patterns in engineered 3D brain microvessels

***Bernabeu, Maria**; Gunnarsson, Celina; Vishnyakova, Maria; Avril, Marion; Nagao, Ryan J.; Taylor, Terrie E.; Seydel, Karl B.; Zheng, Ying; Smith, Joseph D.*

FT4: Cellulose, chitin, and three families of abundant proteins localize to distinct structures in the cyst wall of the eye pathogen Acanthamoeba castellanii

***Magistrado-Coxen, Pamela**; Aqeel, Yousuf; Lopez, Angelo; Haserick, John; Costello, Catherine; Samuelson, John*

COFFEE BREAK

FT5: Stem cell-derived epithelial culture system that supports complete Cryptosporidium parvum development in vitro

***Funkhouser-Jones, Lisa J.**; Wilke, Georgia; Ravindran, Soumya; Wang, Yi; Kuhlenschmidt, Mark; Stappenbeck, Thaddeus; Sibley, L. David*

TT1: Identification of the Plasmodium falciparum var gene interactome with CRISPR-ChIP

***Bryant, Jessica**; Baumgarten, Sebastian; Claës, Aurélie; Scherf, Artur*

TT2: Using humanized malaria parasites to study protein interactions at the erythrocyte cytoskeleton

***Warncke, Jan**; Beilstein, Sabina; Wyss, Matthias; Gabel, Anke; Passecker, Armin; Perez, Lara; Butter, Falk; Beck, Hans-Peter*

TT3: In vivo and in vitro studies of the host-parasite interactions of Spironucleus salmonicida and the Atlantic salmon

***Ástvaldsson, Ásgeir**; Stairs, Courtney; Alfjorden, Anders; Hultenby, Kjell; Jansson, Eva; Svärd, Staffan*

TT4: Data mining and analysis using EuPathDB resources

***Harb, Omar S.**; Warrenfeltz, Susanne; Brunk, Brian; Kissinger, Jessica; Roos, David*

Session II: “I have a gut feeling”: New Approaches for Intestinal Parasites;

Chairs: Katherine Ralston and Josh Beck

Monday, September 10, 2018

9:00 am – 10:10 am

FT1: Genome-wide CRISPR/Cas9 Knockout Screen Identifies Host Genes Important for Cryptosporidium parvum Infection
***Gibson, Alexis R.**; Sateriale, Adam; Striepen, Boris*

FT2: Entamoeba histolytica trogocytosis contributes to acquisition of host cell membrane proteins and protection from lysis by human serum
***Miller, Hannah W.**; Ralston, Katherine S.*

FT3: Genetic evaluation of purine biosynthesis in Cryptosporidium parvum suggests nucleotide uptake from host cell
***Pawlowic, Mattie C.**; Sateriale, Adam; Herbert, Gillian; Matsuda, Rina; Gibson, Alexis; Mead, Jan; Cuny, Gregory; Hedstrom, Lizbeth; Striepen, Boris*

FT4: An NAD+ dependent novel transcription factor controls stage conversion in Entamoeba
***MANNA, DIPAK;** Lentz, Christian; Ehrenkauger, Gretchen; Suresh, Susmitha; Bhatt, Amrita; Singh, Upinder*

TT1: Consistent, comparative and evidence-based genome annotation and re-annotation for the closely-related species, Cryptosporidium parvum, C. hominis and C. tyzzeri *Baptista, Rodrigo P.; Li, Yiran; Sateriale, Adam; Ansell, Brendan; Jex, Aaron; Cotton, James A.; Sanders, Mandy; Brooks, Karen; Tracey, Alan; Berriman, Matthew; Striepen, Boris; Kissinger, Jessica C.*

TT2: Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to Giardia Intestinalis during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation
***Ma'ayeh, Showgy Yasir**; Stadelmann, Britta; Knörr, Livia; Sköld, Karin; Svärd, Staffan*

Coffee Break

Session III: Zombie: Taking Control of the Host

Chairs: Josh Beck and Katherine Ralston

Monday, September 10, 2018

10:30am – 11:30am

Info about meBOP (Omar Harb) and BoP (Photini Sinnis)

FT1: The First Steps Toward Dissecting the "Ins and Outs" of *T. cruzi* During Intracellular Infection
***Chasen, Nathan**; Tarelton, Rick; Etheridge, R. Drew*

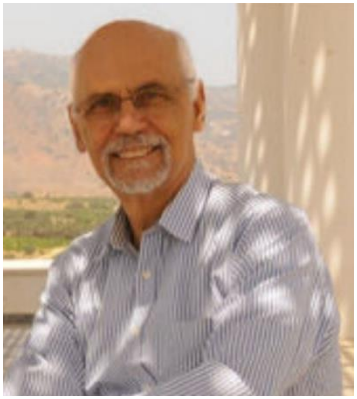
FT2: The Exported FIKK Kinases of P. falciparum Target Key Proteins Involved in Host-cell Modification
***Davies, Heledd M.**; Treeck, Moritz; Belda, Hugo; Broncel, Goska; Tiburcio, Marta*

FT3: Systematic identification of Toxoplasma virulence factors in vivo
***Sangare, Lamba Omar**; Wang, Yifan; Krishnamurthy, Shruthi; Paredes-Santos, Tatiana C; Sidik, Saima M; Lourido, Sebastian; Saeij, Jeroen P.J*

TT1: Targeting Cell Entry as Therapy for Leishmaniasis
***Wetzel, Dawn M.**; Ullah, Imran; Kernen, Rebecca M.; Booshehri, Laela M.; Rhodes, Emma L.; Mamula, Emily T.; Niederstrasser, Hanspeter; Posner, Bruce A.*

TT2: Cryptosporidium parvum exports proteins into the cytoplasm of the epithelial host cell
***Dumaine, Jennifer E.**; Sateriale, Adam; Reddy, Amita; Striepen, Boris*

In Memoriam of Fotis Kafatos



Session IV: Sex and Mosquitoes

Chairs: Jaime Adam-Gallego and Catherine Lavazec

Monday, September 10, 2018

1:00 pm – 2:45 pm

Kirk Deitsch: In Memoriam of Fotis Kafatos

FT1: Mosquito reproductive factors affecting *Anopheles-Plasmodium* interactions
***Perrine Marcenac**, Kristine L. Werling, Duo Peng, Adam South, Evdoxia G. Kakani, Sara N. Mitchell, W. Robert Shaw, Kathleen A. Westervelt, Serge R. Yerbanga, Thierry Lefèvre, Abdoulaye Diabaté, Flaminia Catteruccia*

FT2: A mosquito salivary gland protein partially inhibits Plasmodium sporozoite cell traversal and transmission
***Yang, Jing**; Schleicher, Tyler; Freudzon, Marianna; Rembisz, Alison; Craft, Samuel; Hamilton, Madeleine; Graham, Morven; Mlambo, Godfree; Tripathi, Abhai; Li, Yue; Cresswell, Peter; Sinnis, Photini; Dimopoulos, George; Fikrig, Erol*

FT3: A systematic knockout screen in Plasmodium berghei identifies sex-specific regulators of gametocyte differentiation
***Bushell, Ellen**; Sanderson, Theo; Talman, Arthur; Russell, Andrew; Montandon, Ruddy; Modrzynska, Kasia; Metcalf, Tom; Bronner-Anar, Burcu; Herd, Colin; Kent, Robyn; Girling, Gareth; Schwach, Frank; Rayner, Julian C; Waters, Andrew P; Lawnizcak, Mara K; Billker, Oliver*

FT4: Homeo Domain-like Protein 1 regulates chromatin structure and gene expression during the early stages of P. falciparum sexual development
***Campelo, Riward**; Tong, Xinran; Xie, Wei; Batugedara, Gayani; Cook, Kate; Orchard, Lindsey; Llinas, Manuel; Noble, William; Le Roch, Karine; Patel, Dinshaw; Kafsack, Björn*

FT5: NOT1-G is a novel member of the CAF1/CCR4/NOT complex that is essential for male gametocyte development and female gametocyte fertility in Plasmodium yoelii
***Hart, Kevin**; Walker, Michael; Minns, Allen; Lindner, Scott*

FT6: Plasmodium and Host microbiota: Friend or Foe?
Mukherjee, Debanjan*; Chora, Angelo; Ramiro, Ricardo; Gordo, Isabel; Mota, Maria*

TT1: Breaking the proventricular bottleneck: characterization of genes that enhance trypanosome migration to the tsetse salivary glands
Casas-Sanchez, Aitor*; Lopez-Escobar, Lara; Cansado-Utrilla, Cintia; Haines, Lee; Walrad, Pegine; Acosta-Serrano, Alvaro*

TT2: Nutritional Supplementation Enhances Innate Immune Response Against Plasmodium Liver Stage Parasites
Meireles, Patricia*; Bras, Daniela; Mendes, Antonio; Fontinha, Diana; Andrade, Carolina; Prudencio, Miguel*

Coffee Break

Power hour: 4pm-5pm Jayne Raper and Nina Papvasilliou moderated by Tamar Haspel (Journalist-Washington Post)
An informal session for all attendees to discuss challenges women face in science: Data on disparity will be presented, and then assigned to breakout groups to discuss and solicit solutions for group discussion.

In Memoriam of Ruth Nussenzweig

Session V: I like to Move It

Chairs: Aoife Heaslip and Hangjun Ke

Tuesday, September 11, 2018

9:00 am – 11:35 am

Elizabeth H. Nardin: In Memoriam of Ruth Nussenzweig

FT1: Repeat region of the circumsporozoite protein has a functional role in Plasmodium sporozoite motility
***Balaban, Amanda**; Mitra, Jaba; Gregory, Jason; Vartak, Natasha; Sinnis-Bourozikas, Ariadne; Shears, Melanie; Ha, Taekjip; Frischknecht, Friedrich; Sinnis, Photini*

FT2: An alveolate conserved mechanism is implicated in rhoptries secretion event during Apicomplexa invasion
***Aquilini, Eleonora**; Dos Santos Pacheco, Nicolas; Suarez, Catherine; Maynadier, Marjorie; Lebrun, Maryse*

FT3: Combining Reverse Genetics and Three-dimensional Electron Microscopy to Dissect Plasmodium knowlesi Invasion of Red Blood Cells
***Hart, Melissa**; Mohring, Franziska; Charleston, James; Thomas, James; Saibil, Helen; Moon, Robert*

FT4: It Takes Two to Tango: the P52/P36 Hepatocyte Invasion Complex
***Arredondo, Silvia A.**; Swearingen, Kristian E.; Martinson, Thomas; Steel, Ryan; Dankwa, Dorender A.; Harupa, Anke; Camargo, Nelly; Betz, William; Vigdorovich, Vladimir; Oliver, Brian G.; Ishino, Tomoko; Kangwanrangsan, Niwat; Sather, Noah; Mikolajczak, Sebastian; Vaughan, Ashley M.; Torii, Motomi; Moritz, Robert L.; Kappe, Stefan H. I.*

TT1: DOC2 domain proteins in Toxoplasma gondii Ca2+-dependent secretion
***Tagoe, Daniel A.**; Coleman, Bradley; Stoneburner, Emily; Drozda, Allison; Coppens, Isabelle; Gubbels, Marc-Jan*

TT2: Blood donor variability as a modulatory factor in Plasmodium falciparum invasion phenotyping assays
***Thiam, Laty G.**; Aniweh, Yaw; kusi, Kwadwo A.; Niang, Makhtar; Gwira, Theresa M.; Awandare, Gordon A.*

COFFEE BREAK

FT5: Lysosome exocytosis by hepatocytes is pivotal to Plasmodium infection

Vijayan, Kamalakannan*; Cestari, Igor; Mast, Fred; Glennon, Elizabeth; Kain, Heather; Brokaw, Alyssa; Aitchinson, John; Stuart, Kenneth; Kaushansky, Alexis*

FT6: The Great Escape: investigating the role of SERA6 in malaria parasite egress from the red blood cell

Tan, Michele Ser Ying*; Thomas, James A.; Borg, Aaron; Howell, Steven; Snijders, Ambrosius P.; Blackman, Michael J.*

FT7: Malaria Parasite Egress Program: Vacuolar Rounding, Exonemal Activation of Membrane Rupture and Degradation

Glushakova, Svetlana*; Beck, Josh R; Garten, Matthias; Busse, Brad L.; Nasamu, Armiyaw S.; Tenkova-Heuser, Tatyana; Heuser, John; Goldberg, Daniel; Zimmerberg, Joshua*

TT3: A novel role for EXP2 in invasion of Plasmodium sporozoites

Mello-Vieira, Joao*; de Koning-Ward, Tania; Mota, Maria; Zuzarte-Luís, Vanessa*

TT4: Impact of malaria-protective glycophorin polymorphism on Plasmodium falciparum invasion

Kariuki, Silvia N.*; Marin-Menendez, Alejandro; Leffler, Ellen; Band, Gavin; Rockett, Kirk; Macharia, Alex; Makale, Johnstone; Nyamu, Wilfred; Ndung'u, Francis; Kwiatkowski, Dominic; Williams, Thomas; Rayner, Julian*

TT5: Comparative assessment of PbSLTRiP (Sporozoite and Liver stage expressed Tryptophan Rich Protein) peptides as vaccine candidates against Plasmodium berghei in mice.

Quadiri, Afshana*; Singh, Agam Prasad*

Session VI: Bad to the Bone

Chairs: Prakash Srinivasan and Taco Kooij

Tuesday, September 11, 2018

1:00 pm – 1:50 pm

FT1Stuck on You: The Plasmodium basal complex is required for proper daughter cell segmentation
Rudlaff, Rachel M.*; Streva, Vincent A.; Dvorin, Jeffrey D.*

FT2: The dually localised actin regulator TgCAP is important for controlling a subset of actin-dependent processes, including cell-cell communication, motility and organisation within the host-cell
Hunt, Alex*; Kent, Robyn; Carmeille, Romain; Russell, Matt; Wagener, Jeanette; Heaslip, Aoife; Ward, Gary; Treeck, Moritz*

FT3: Keeping in shape: The role of GAPM proteins in maintaining the structure of Toxoplasma gondii
Harding, Clare*; Ho Kang, Joon; Meissner, Markus; Lourido, Sebastian*

TT1: TgMyoF is an organizer of the endosome-like compartment in Toxoplasma gondii
Carmeille, Romain*; Heaslip, Aoife*

Coffee Break

Session VII: Fission and Fusion

Chairs: Taco Kooij and Prakash Srinivasan

Tuesday, September 11, 2018

2:20 pm – 3:30 pm

FT1: Toward deciphering Toxoplasma. gondii endocytosis during gliding

***Gras, Simon**; Jimenez-Ruiz, Elena; Lemgruber, Leandro; Klinger, Christen; Meissner, Markus*

FT2: Inositol Pyrophosphates Control Phosphate Release from Acidocalcisomes of Trypanosoma brucei and Vacuoles of Saccharomyces cerevisiae

***Potapenko, Evgeniy**; Cordeiro, Ciro; Huang, Guozhong; Storey, Melissa; Jessen, Henning; Starai, Vincent; Docampo, Roberto*

FT3: A bacterial complex is required for plastid integrity in P. falciparum

***Florentin, Anat**; Stephens, Dylon; Muralidharan, Vasant*

FT4: A large conductance ion channel on the malaria parasite digestive vacuole

***Saggu, Gagandeep S.**; Desai, Sanjay*

TT1: CATHEPSIN L (TBCATL) PROCESSING AND POST-GOLGI SORTING TO THE LYSOSOME IN AFRICAN TRYPANOSOMES

***Koeller, Carolina M.**; Bangs, James D.*

TT2: Characterization of vesicular systems of artemisinin resistance suggest their roles in trafficking and virulence, dependent and independent of Kelch13

***Suresh, Niraja**; Khair, Maisha; Coppens, Isabelle; Bhattacharjee, Souvik; Mbengue, Alassane; Ghorbal, Mehdi; Haldar, Kasturi*

EupathDB open community meeting: 3.30-5pm

- New datasets and analysis tools in EuPathDB
- Underappreciated resources: for analysis of your own data, cross-species phylogenetic inference, metabolic pathway mapping, phenotypic analysis, etc
- How can we do a better job of outreach, e.g. how to best ensure awareness of the above?
- Plans for renewal of the EuPathDB contract

Session VIII: Clubbed to Death

Chairs: Jennifer Guler and Selina Bopp

Wednesday, September 12, 2018

9:00 am – 11:30 am

Keynote Lecture: 5.30-7.00 pm

FT1: TgBDP3 is a member of the Toxoplasma TFIID complex and a target of the anti-parasitic bromodomain inhibitor I-BET151
***Jeffers, Vicki**; Hanquier, Jocelyne; Sullivan Jr, William J.*

FT2: Delayed death in the malaria parasite: prenylation dependant disruption of intracellular trafficking
***Kennedy, Kit**; Cobbold, Simon; Spillman, Natalie; Hanssen, Eric; Blanch, Adam; Namvar, Arman; Tilley, Leann; McConville, Malcolm; Ralph, Stuart*

FT3: Antimalarial N-Myristoyl Transferase inhibitors: a resistant mutant parasite provides insights into inhibitor binding mode
***Schlott, Anja**; Mayclin, Stephen; Reers, Alexandra; Coburn-Flynn, Olivia; Bell, Andrew; Green, Judith; Knuepfer, Ellen; Charter, David; Lyons-Abbott, Sally; Staker, Bart; Myler, Peter; Chung, Chun-wa; Fidock, David; Tate, Edward; Holder, Anthony*

TT1: Mutations in the actin-binding protein PfCoronin confer resistance to Artemisinin in West African Plasmodium falciparum isolates
Sharma, Aabha

TT2: Mind the traffic: a role for trafficking in the cell stress response and artemisinin resistance in P. falciparum
***Henrici, Ryan**; Zoltner, Martin; Hart, Melissa; Edwards, Rachel; van Schalkwyk, Don; Baker, David; Moon, Rob; Odom John, Audrey; Field, Mark; Sutherland, Colin*

TT3: Characterizing known translation inhibitors and drug candidates via Plasmodium falciparum whole cell extracts vs. S35 methionine incorporation: separating true 80S ribosome inhibition from artifact
***Sheridan, Christine**; Garcia, Valentina; Ahyong, Vida; DeRisi, Joseph*

COFFEE BREAK

FT4: Development of drug-resistant Naegleria fowleri to identify target of novel class of amidines
Colon, Beatrice L.*; de Paula Baptista, Rodrigo; Farahat, Abdelbasset; Boykin, David; Kyle, Dennis*

FT5: Unravelling chromosomal segregation in Trypanosomatid parasites: the nucleoporin TbMLP1 plays an essential role in the maintenance of ploidy
STERKERS, Yvon*; YAGOUBAT, Akila; STANOJCIC, Slavica; BERRY, Laurence; BRYANT,Jessica; CROBU, Lucien; KUK, Nada; BASTIEN, Patrick; SCHERF, Artur*

FT6: Investigation of arginine methylation function in Leishmania (V.) braziliensis growth and infection
Lorenzon, Lucas*; Diniz, Juliana; Ferreira, Tiago; Walrad, Pegine; Cruz, Angela*

TT4: Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi
Berná, Luisa*; Rodriguez, Matias; Chiribao, Maria Laura; Parodi-Talice, Adriana; Pita, Sebastián; Rijo, Gastón; Alvarez-Valin, Fernando; Robello, Carlos*

TT5: Functional characterization of mitochondrial translation components in the early diverging eukaryote Toxoplasma gondii
Lacombe, Alice*; Tottey, J.; Ovcuarujiva, J.; Courjol, F.; Gissot, M.; Sheiner, L.*

TT6: Antimalarial Drug Target PfATP4 is Present in Parasite Plasma Membrane as a Large Complex
ramanathan, Aarti*; Vaidya, Akhil; Bhatnagar, Suyash; Morrisey, Joanne*

Keynote Lecture: The Hidden Life of African Trypanosomes
Stephen L Hajduk, Department of Biochemistry & Molecular Biology
University of Georgia

Session IX: Switch It On, Switch Off

Chairs: Galadriel Hovel-Minor and Sumiti Vinayak

Thursday, September 13, 2018

9:00 am – 10:00 am

FT1: Role of the eIF4E1-4EIP complex in gene repression during differentiation of trypanosomes
***Marucha, Kevin**; Egler, Franziska; Terrao, Monica; Mugo, Elisha; Braun, Johanna; Droll, Dorothea; Minia, Igor;, Christine*

FT2: Extensive m6A RNA methylation dynamically modulates gene expression in Plasmodium falciparum
***Baumgarten, Sebastian**; Bryant, Jessica; Sinha, Ameya; Reyser, Thibaud; Dedon, Peter; Preiser, Peter; Scherf, Artur*

FT3: Investigation of the Not1 proteins in regulating gene expression in Plasmodium falciparum
***Liu, Ying**; Featherstone, Mark; Bozdech, Zbynek*

FT4: Identification of PfAP2-HS as the master regulator of the heat shock response in Plasmodium falciparum
***Tintó-Font, Elisabet**; Michel-Todó, Lucas; Cortés, Alfred*

COFFEE BREAK

Session X: The best for last

Chairs: Sumiti Vinayak and Galadriel Hovel-Minor

Thursday, September 13, 2018

10:30 am – 11:30 am

FT5: Structural studies of a novel SET-domain containing lysine methyltransferase that regulates motility of Toxoplasma gondii
***Pivovarova, Yulia**; Lesigang, Johannes; Hu, Ke; Liu, Jun; Dong, Gang*

FT6: Evolution of mitochondrial TAT translocases illustrates the demise of bacterial protein transport machines in mitochondria
***Markéta, Petru**; Wideman, Jeremy; Moore, Kristoffer; Alcock, Felicity; Palmer, Tracy; Doležal, Pavel*

FT7: miR-34c plays a key role during host-parasite infections in Theileria-infected macrophages and Plasmodium falciparum-infected red blood cells by targeting PRKAR2B
***Haidar, Malak**; Ben-Rached, Fathia; Langsley, Gordon; Pain, Arnab*

FT8: Pentatricopeptide repeat factor functions as poly(A) binding protein in mitochondria of T. brucei
***Mesitov, Mikhail**; Yu, Tian; Suematsu, Takuma; Zhang, Liye; Huang, Lan; Aphasizheva, Inna*

Passing the Torch:

Meet the Organisers of MPM 2019-2021

Poster Session A

Monday, September 10, 2018

7:00 pm – 9:00 pm

Poster Numbers 1 - 74

1: Identification of the Plasmodium falciparum var gene interactome with CRISPR-ChIP
***Bryant, Jessica M.**; Baumgarten, Sebastian; SCHERF, Artur*

2: Using humanized malaria parasites to study protein interactions at the erythrocyte cytoskeleton
***Warncke, Jan**; Beilstein, Sabina; Wyss, Matthias; Gabel, Anke; Passecker, Armin; Perez, Lara; Butter, Falk; Beck, Hans-Peter*

3: In vivo and in vitro studies of the host-parasite interactions of Spironucleus salmonicida and the Atlantic salmon
***Ástvaldsson, Ásgeir**; Stairs, Courtney; Alfjorden, Anders; Hultenby, Kjell; Jansson, Eva; Svärd, Staffan*

4: Data mining and analysis using EuPathDB resources
***Harb, Omar S.**; Warrenfeltz, Susanne; Brunk, Brian; Kissinger, Jessica; Roos, David*

5: Consistent, comparative and evidence-based genome annotation and re-annotation for the closely-related species, Cryptosporidium parvum, C. hominis and C. tyzzeri
***Baptista, Rodrigo P.**; Li, Yiran; Sateriale, Adam; Ansell, Brendan; Jex, Aaron; Cotton, James A.; Sanders, Mandy; Brooks, Karen; Tracey, Alan; Berriman, Matthew; Striepen, Boris; Kissinger, Jessica C.*

6: Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to Giardia Intestinalis during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation
***Ma'ayeh, Showgy Yasir**; Stadelmann, Britta; Knörr, Livia; Sköld, Karin; Svärd, Staffan*

7: Targeting Cell Entry As Therapy for Leishmaniasis
***Wetzel, Dawn M.**; Ullah, Imran; Kernen, Rebecca M.; Booshehri, Laela M.; Rhodes, Emma L.; Mamula, Emily T.; Niederstrasser, Hanspeter; Posner, Bruce A.*

8: Cryptosporidium parvum exports proteins into the cytoplasm of the epithelial host cell
***Dumaine, Jennifer E.**; Sateriale, Adam; Reddy, Amita; Striepen, Boris*

9: Breaking the proventricular bottleneck: characterization of genes that enhance trypanosome migration to the tsetse salivary glands
***Casas-Sanchez, Aitor**; Lopez-Escobar, Lara; Cansado-Utrilla, Cintia; Haines, Lee; Walrad, Pegine; Acosta-Serrano, Alvaro*

10: Nutritional Supplementation Enhances Innate Immune Response Against Plasmodium Liver Stage Parasites
***Meireles, Patricia**; Bras, Daniela; Mendes, Antonio; Fontinha, Diana; Andrade, Carolina; Prudencio, Miguel*

11: Characterization of the perforin-like protein family in Babesia sp.
***Paoletta, Martina S.**; Jaramillo Ortiz, José; López Arias, Ludmila; Montenegro, Valeria; Suarez, Carlos; Farber, Marisa; Wilkowsky, Silvina*

12: Pathogen screening from soft and hard ticks isolated from different species in Chihuahua, Mexico
***Adame-Gallegos, Jaime R.**; Beristain-Ruiz, Diana M.; Medrano-Bugarini, Raúl A.; Lira-Amaya, José J.; Rodríguez-Alarcón, Carlos A.; Figueroa-Millán, Julio V.; Rivera-Chavira, Blanca E.*

13: Allosteric Site Inhibitor Disrupting Auto-activation of Cysteine Proteases of Plasmodium falciparum
***Pant, Akansha**; kumar, Rajendra; Wani, Naiem; Verma, Sona; Sharma, Ruby; Pande, Veena; Saxena, Ajay; Dixit, Rajnikant; Rai, Rajkishore; Pandey, Kailash*

14: Molecular dissection of Plasmodium falciparum importin a protein towards its assessment as a potential drug target
***Dey, Vishakha**; Patankar, Swati*

15: Molecular dissection of Plasmodium falciparum importin a protein towards its assessment as a potential drug target
***Dey, Vishakha**; Patankar, Swati*

16: Identification of cryptic stator subunits from an apicomplexan ATP synthase
***Huet, Diego**; Rajendran, Esther; Van Dooren, Giel; Lourido, Sebastian*

17: Unveiling crucial interactions between malarial cysteine proteases, falcipains and their natural substrate and macromolecular inhibitor
***Pasupureddy, Rahul**; Verma, Sonia; Pant, Akansha; Sharma, Ruby; Seshadri, Sriram; Saxena, Ajay; Dixit, Rajnikant; Pandey, Kailash*

18: Evidence of altered liver function and cytokine response profiles in pregnant women with malaria and chronic hepatitis B
***Anabire, Nsoh Godwin**; Aryee, Paul; Abdul-Karim, Abass; Quaye, Osbourne; Awandare, Gordon; Helegbe, Gideon*

19: Identification and characterization of Toxoplasma genes that determine fitness in interferon gamma-stimulated human cells.
***Krishnamurthy, Shruthi**; Parades Santos, Tatiana; Sangare, Lamba Omar; Wang, Yifan; Sidik, Saima; Lourido, Sebastian; Saeij, Jeroen P.*

20: P-cyclins are master regulators of the Toxoplasma intermediate life cycle
***Suvorova, Elena S.**; Alvarez, Carmelo A.; White, Michael W.*

21: High Cysteine Proteins play a major role during Giardia intestinalis interactions with host intestinal epithelial cells
***Peirasmaki, Dimitra**; Ferella, Marcela; Höppner, Marc; Campos, Sara; Ankarklev, Johan; Stadelmann, Britta; Grabherr, Manfred; Svärd, Staffan*

22: Lipoate dependent enzymes of Plasmodium falciparum
***Nair, Sethu C**.; Prigge, Sean*

23: Toxoplasma proteins involved in nutrient acquisition across the parasitophorous vacuole membrane during acute and chronic stages
***Paredes Santos, Tatiana**; Krishnamurthy, Shruthi; Sangare, Lamba; Wang, YiFan; Cardoso Barros, Patricio; Attias, Marcia; Lourido, Sebastian; Sidik, Saima; Gold, Daniel; P Saeij, Jeroen*

24: Developing a Detection Method for Trypanosoma congolense in Various Mouse Tissue Types
***Pangburn, Sarah**; Verdi, Joey; Raper, Jayne*

25: Identification of metabolic pathways that regulate sexual differentiation in P. falciparum.
***Sollelis, Lauriane**; Straub, Timothy J; De Niz, Mariana; Laffitte, Marie-Claude; Brancucci, Nicolas M B; Barrett, Michael; Neafsey, Daniel E; Marti, Matthias*

26: Structural studies of the interaction between FPC3 and FPC4, two essential components of the flagellar pocket collar in Trypanosoma brucei
***Majneri, Paul**; Dong, Gang; Pivovarova, Yulia; Landrein, Nicolas; Lesigang, Johannes; Robinson, Derrick; Bonhivers, Mélanie*

27: Lathosterol oxidase is important for acid resistance and lipophosphoglycan synthesis in Leishmania major
***Ning, Yu**; Hsu, Fong-Fu; Zhang, Kai*

28: High throughput screening to identify selective proteasome inhibitors as new antimalarials with a novel mode of action
***Mata-Cantero, Lydia**; Cortés, Álvaro; García, Mercedes; Xie, Stanley; Gillett, David; Gamo, Javier; Tilley, Leann; Gómez, María G.*

29: A Toxoplasma Oxygen Sensing Protein Mediates Cellular Responses to Hyperoxic Stress and is Required for Tissue Specific Dissemination
***Blader, Ira;** Florimond, Celia; Cordonnier, Charlotte; Taujale, Rahil; va der Wel, Hanke; Kannan, Natarajan; West, Chris*

30: Identification of regulatory factors that determine Toxoplasma bradyzoite to merozoites conversion
***Arranz-Solís,** David; Mukhopadhyay, Debanjan; Saeij, Jeroen*

31: Unconventional ribosomes with highly fragmented rRNAs in malaria parasite mitochondria
***Ke, Hangjun; Dass,** Swati; Mulaka, Maruthi; Gutierrez-Vargas, Cristina; Mather, Michael; Dvorin, Jeffrey; Frank, Joachim; Vaidya, Akhil*

32: Sterol synthesis has major impact on the mitochondrial physiology and stress response in Leishmania major.
***Mukherjee, Sumit; Xu**, Wei; Zhang, Kai*

33: Development of CRISPR/Cas9-mediated disruption systems in Giardia lamblia
Sun, Chin-Hung*; Lin, Zi-Qi; Gan, Soo-Wah; Tung, Szu-Yu; Ho, Chun-Che; Su, Li-Hsin*

34: A myeloid leukemia factor-like protein involved in encystation-induced protein metabolism in Giardia lamblia
Sun, Chin-Hung*; Wu, Jui-Hsuan; Lin, Zi-Qi,; Su, Li-Hsin; Gan, Soo-Wah; Liao, Jo-Yu; Tung, Szu-Yu*

35: PfSR1 is essential for DNA damage repair and resistance to artemisinin in Plasmodium falciparum
Dzikowski, Ron*; Singh, Brajesh; Goyal, Manish; Siman-Tov, Karina; Kaufman, Yotam; Eshar, Shiri*

36: Structural characterisation of the merozoite surface protein-1 using recombinant proteins from different Plasmodium strains
Marzluf, Tanja*; Bujard, Hermann*

37: The Role of BEI Resources as a Centralized Repository for Parasitology Research
Molestina, Robert

38: The Proteome of Malaria Parasite Merosomes and Liver Stage Merozoites
Shears, Melanie J*.; Nirujogi, Raja; Swearingen, Kristian; Renuse, Santosh; Mishra, Satish; Mortiz, Robert; Pandey, Akhilesh; Sinnis, Photini*

39: Thymidylate synthase validation as antimalarial target using CRISPR-Cas9
Moliner-Cubel, Sonia*; Palomo-Diaz, Sara; Franco-Hidalgo, Virginia; Winzeler, Elizabeth; Gomez-Lorenzo, Maria; Gamo, Javier*

40: Defining a transcriptional repressor complex controlling tissue cyst development in Toxoplasma gondii
Srivastava, SandeepSandeep*; Huang, Sherri; Holmes, Michael J.; Radke, Joshua B.; Hong, David; Liu, Ting-Kai; White, Michael W.; Sullivan Jr, William J.*

41: Turnover ofof VSG In Trypanosoma BruceiBrucei
Garrison, Paige*; Umaer, Khan; Bush, Peter; Bangs, Jay*

42: Proximity-dependent biotin labeling reveals the spatial organization of the Plasmodium DOZI/CITH/ALBA complex
Rios, Kelly*; Lindner, Scott*

43: Electron microscopy and cross-linking mass spectrometry studies of RNA editing complexes in T. brucei
Wang, Hong*; Havugimana, Pierre; Huang, Lan; Emili, Andrew; Zhou, Z. Hong; Afasizhev, Ruslan*

44: Mechanism of action of a novel anti-trypanosomal compound class identified from phenotypic screen
Palkar, Rima*; Gould, Matthew; Johnson, Nila; Ritchie, Ryan; Schmidt, Remo; Kaiser, Marcel; Maeser, Pascal; Jiricek, Jan; Diagana, Thierry; Barrett, Michael; Rao, Srinivasa*

45: Identification of novel druggable targets in Plasmodium falciparum using chemical mutagenesis
Clements, Rebecca L*.; Streva, Vincent; Dumoulin, Peter; Huang, Weigang; Burleigh, Barbara; Zhang, Qisheng; Dvorin, Jeffrey*

46: Toxoplasma effector GRA15 enhances inflammasome induced cell death in human primary fibroblasts
Mukhopadhyay, Debanjan*; Sangare, Lamba Omar; Saeij, Jeroen*

47: Unravelling the mode of action of a novel class of antimalarials
DeDe Vries, Laura*; Kooij, Taco WA; Verhoef, Julie; Allman, Erik; Jansen, Patrick; Koolen, Karin; Bolscher, Judith; Vos, Martijn; Josling, Gabrielle; Llinás, Manuel; Sauerwein, Robert; Schalkwijk, Joost; Dechering, Koen*

48: Deciphering STEVOR adhesive properties in Plasmodium falciparum gametocyte-infected erythrocytes
Neveu, Gaëlle*; dupuy, florian; Ladli, Meriem; Barbieri, Daniela; Naissant, Bernina; Richard, Cyrielle; Bachmann, Anna; Bischoff, Emmanuel; Verdier, Frédérique; Lavazec, Catherine*

***49*: Protozoa are a global burden - and need new treatments and novel drug targets**
Mbekeani, Alison

50: Characterization of multiple MORN-repeat proteins in Toxoplasma gondii and their potential roles in phosphoinositide/calcium signalling
Ke, HuilingHuiling*; Koreny, Ludek; Barylyuk, Konstantin; Waller, Ross*

51: Transcription initiation defines kinetoplast RNA boundaries
Suematsu, Takuma*; Sement, François M.; Yu, Tian; Zhang, Liye; Huang, Lan; Afasizheva, Inna; Afasizhev, Ruslan*

52: Localization and role of a mechanosensitive channel in the procyclic form of Trypanosoma brucei
Hernandez, Monica*; Nguyen, Kristy; Dave, Noopur; Jimenez, Veronica*

53: Analysis of mechanosensation-dependent functions in Trypanosoma cruzi
Fonbuena, Joshua*; Feldman, Marc; Tiwari, Megna; Jimenez, Veronica*

54: Role of a Secreted Effector of Toxoplasma gondii in Modulating the Host Cell Cycle
Pierre-Louis, Edwin*; Etheridge, Menna; Etheridge, R. Drew*

55: Mutational analysis of the leucine zipper in APOL1 and the effect on channel formation
Lee, Penny; Schaub, *Charles; Racho-Jansen, Alisha; Kim, Ryan; Raper, Jayne*

56: Identifying an APOLI Binding Partner Through SRA-Specific Antibodies *Ko, Daphne; Verdi**, Joey; Leidich, Raymond; Huang, Tammy; Raper, Jayne*

57: The Promiscuous IgM Antibodies Associated with Trypanosome Lytic Factors
Verdi, Joey; Zipkin*, Ronnie; Sternberg, Jeremy; Thomson, Russell; Raper, Jayne*

58: Spatial organization of the blood stage parasitophorous vacuole of Plasmodium falciparum
Garten, Matthias*; Beck, Josh R; Glushakova, Svetlana; Bleck, Christopher K.E.; Heuser, John; Tenkova-Heuser, Tatyana; Roth, Robyn; Zimmerberg, Joshua; Goldberg, Daniel*

59: Increased expression glucosylceramide transferase in Giardia interferes with cyst production and parasite infectivity
Enriquez, Vanessa; *Grajeda, Brian; De Chatterjee, Atasi; Diaz-Martinez, Laura; Das, Siddhartha*

60: Characterizing Genes Involved in Trogocytosis (cell-nibbling) in Entamoeba histolytica
Feeney, Shea, E.; *Ralston, Katherine, S.*

61: Developing a Genome-wide RNAi Knock Down Screen in Entamoeba histolytica
***Bettadapur, Akhila**; Ralston, Katherine S.*

62: Epistasis studies reveal functional redundancy among calcium-dependent protein kinases in motility and invasion of malaria parasites
***Fang, Hanwei;** Gomes, Ana Rita; Klages, Natacha; Pino, Paco; Maco, Bohumil; Walker, Eloise; Zenonos, Zenon; Angrisano, Fiona; Baum, Jake; Doerig, Christian; Baker, David; Billker, Oliver; Brochet, Mathieu*

63: Systematic Identification of host factors that regulate Plasmodium LS infection
***Probst, Alexandra S.;** Lewis, Adam; Vijayan, Kamalakannan; Parks, K. Rachael; Arang, Nadia; Kaushansky, Alexis*

64: ERAD and disposal of GPI-Anchored proteins in African trypanosomes
***Bangs, James D**.; Koeller, Carolina M.; Tiengwe, Calvin*

65: Detection and isolation of Trypanosoma brucei extracellular vesicles during an active infection
***Cipriano, Michael J.;** Palmer, Margot; Hajduk, Steven*

66: The chromatin-bound proteome of the human malaria parasite, Plasmodium falciparum
Williams, Desiree

67: A secreted kinase regulates membrane structures of the Toxoplasma parasitophorous vacuole
***Beraki, Tsebaot**; Hu, Xiaoyu; Young, Joanna; O'Shaughnessy, William; Treeck, Moritz; L Reese, Michael*

68: Unexpected Link Between Lipid Droplets, Cell Morphology, and Glucose Metabolism
***Pazzo, Kyle**; Paul, Kimberly; Raja, Sripriya; Featherstone, Ellen; Adamson, Michelle; Croft, Lanie*

69: A unique GCN5 histone acetyltransferase complex mediates gene transcription in Plasmodium falciparum
***Miao, JUN**; li, xiaolian; Liang, Xiaoying; Sebastian, Aswathy; Cui, Liwang*

70: Epigenetic Mechanisms and Histone Modifications in Giardia lamblia Antigenic Variation
***Orozco, Daniel**; Garlapati, Srinivas*

71: Using Riboswitch to Control Internal Ribosome Entry Site Mediated Translation Initiation in Giardiavirus
McMahan, Timothy S.

72: Developing a scalable functional genetics pipeline for the malaria parasite, Plasmodium falciparum
Esherick, Lisl Y*.; McGuffie, Bryan A.; Nasamu, Sebastian; Dey, Vishakha; Dvorin, Jeffrey D.; Niles, Jacquin C.*

73: Novel Method to Study Multigene Families in Plasmodium falciparum
Omelianczyk, Radoslaw*; loh, han ping; Preiser, Peter*

74: Engineering a self-targeting entry inhibitor for vectored malaria prophylaxis
SRINIVASAN, PRAKASH*; Xiao, Shuhao; Pandey, Rajeev; Bell, Cameron; Verma, Garima; Ketner, Gary*

Poster Session B

Tuesdsay, September 11, 2018

7:00 pm – 9:00 pm

Poster Numbers 75 - 149

75: DOC2 domain proteins in Toxoplasma gondii Ca2+-dependent secretion
Tagoe, Daniel A.; *Coleman, Bradley; Stoneburner, Emily; Drozda, Allison; Coppens, Isabelle; Gubbels, Marc-Jan*

76: Blood donor variability as a modulatory factor in Plasmodium falciparum invasion phenotyping assays
Thiam, Laty G*.; Aniweh, Yaw; kusi, Kwadwo A.; Niang, Makhtar; Gwira, Theresa M.; Awandare, Gordon A.*

77: A novel role for EXP2 in invasion of Plasmodium sporozoites
Mello-Vieira, Joao*; de Koning-Ward, Tania; Mota, Maria; Zuzarte-Luís, Vanessa*

78: Impact of malaria-protective glycophorin polymorphism on Plasmodium falciparum invasion
Kariuki, Silvia N*.; Marin-Menendez, Alejandro; Leffler, Ellen; Band, Gavin; Rockett, Kirk; Macharia, Alex; Makale, Johnstone; Nyamu, Wilfred; Ndung'u, Francis; Kwiatkowski, Dominic; Williams, Thomas; Rayner, Julian*

79: Comparative assessment of PbSLTRiP (Sporozoite and Liver stage expressed Tryptophan Rich Protein) peptides as vaccine candidates against Plasmodium berghei in mice.
Quadiri, Afshana*; Singh, Agam Prasad*

80: TgMyoF is an organizer of the endosome-like compartment in Toxoplasma gondii
Carmeille, Romain*; Heaslip, Aoife*

81: Cathepsin L (TBCATL) Processing And Post-Golgi Sorting To The Lysosome In African Trypanosomes
Koeller, Carolina M*.; Bangs, James D.*

82: Characterization of vesicular systems of artemisinin resistance suggest their roles in trafficking and virulence, dependent and independent of Kelch13
Suresh, Niraja*; Khair, Maisha; Coppens, Isabelle; Bhattacharjee, Souvik; Mbengue, Alassane; Ghorbal, Mehdi; Haldar, Kasturi*

83: Rapid, iterative and scalable genome editing in zoonotic malaria parasite Plasmodium knowlesi
Mohring, Franziska*; Hart, Melissa N.; Rawlinson, Thomas; Henrici, Ryan; Patel, Avnish; Baker, David; Sutherland, Colin; Draper, Simon; Moon, Robert W*

84: A patatin-like phospholipase is involved in gametocytogenesis of the human malaria parasite Plasmodium falciparum
***Flammersfeld, Ansgar**; Lang, Christina; Flieger, Antje; Sollelis, Lauriane; Marti, Matthias; Ngwa, Che Julius; Pradel, Gabriele*

85: Analysis of Plasmodium berghei infection and occupancy of Anopheles stephensi salivary glands
***Wells, Michael B**.; Villamor, Jordan; Andrew, Deborah*

86: Plasmodium falciparum Phosphatidylinositol 3'-Kinase is a Novel Target for the Development of Next-Generation Artemisinin Combination Therapies
***Iyengar, Kalpana**; Siriwardana, Amila; Hassett, Matthew; Eastman, Richard; Thomas, Craig; Roepe, Paul*

87: Plasmodium sERAD is essential for apicoplast maintenance
***Rajaram, Krithika**; Prigge, Sean*

88: The news about ISC system in the mitosomes of Giardia intestinalis
***Motyckova, Alzbeta**; Stairs, Courtney; Najdrova, Vladimira; Voleman, Lubos; Dolezal, Pavel*

89: Visualizing and identifying secreted effectors from Toxoplasma gondii bradyzoites
***Mayoral, Joshua**; Tu, Vincent; Tomita, Tadakimi; Ma, Yanfen; Weiss, Louis*

90: Identifying the mechanism of action of Tartrolon E, a broad spectrum anti-apicomplexan compound
***Bowden, Gregory**; Driskell, Iwona; Nepveux, Felix; Lin, Zhenjian; Schmidt, Eric; Schafer, Deborah; Riggs, Michael; O'Connor, Roberta*

91: Polyunsaturated fatty acids promote Plasmodium falciparum gametocytogenesis
***Tanaka, Takeshi**; Tokuoka, Suzumi; Nakatani, Daichi; Hamano, Fumie; Kawazu, Shin-ichiro; Wellems, Thomas; Kita, Kiyoshi; Shimizu, Takao; Tokumasu, Fuyuki*

92: PfEMP1 proteins binding non-immune IgM are common among Plasmodium falciparum parasites
***Quintana, Maria del Pilar**; Ditlev, Sisse; Ecklu-Mensah, Gertrude; Hviid, Lars; Lopez-Perez, Mary*

93: Identification of Toxoplasma gondii genes that determine fitness in interferon gamma-stimulated rodent macrophages
Wang, Yifan*; Sangaré, Lamba Omar; Krishnamurthy, Shruthi; Paredes-Santos, Tatiana; Sidik, Saima; Lourido, Sebastian; Saeij, Jeroen*

94: An essential Flagellum Pocket Collar protein of Trypanosoma brucei that localises to the centrosome when expressed in mammalian cells
Reix, Christine E*; Cayrel, Anne; Florimond, Celia; Landrein, Nicolas; Dacheux, Denis; Morriswood, Brooke; Robinson, Derrick*

95: Heme Activation of Artemisinin Antimalarial Drugs
Heller, Laura*; Roepe, Paul*

96: Low mutation rate during Eimeria maxima precocity selection
Hu, Dandan*; Wang, Chaoyue; Liu, Xianyong; Suo, Xun*

97: High-level resistance to Plasmodium falciparum cytochrome B inhibitors maps to residues 126 and 268
Lane, Kristin D*.; Mu, Jianbing; Liu, Jinghua; Windle, Sean T.; Sun, Peter D.; Wellems, Thomas E.*

98: Exploring the regulatory role of the transcription factor ApiAP2 in Toxoplasma gondii sexual development
Wang, Chaoyue*; Tang, Xinming; Hu, Dandan; Wang, Si; Liu, Xianyong; Suo, Xun*

99: Structure, function and dynamics of minimalist mitochondria of Giardia intestinalis.
Dolezal, Pavel*; Pyrihova, Eva; Motyckova, Alzbeta; Voleman, Lubos; Najdrova, Vladimira; Wandyszewska, Natalia*

100: Dissecting the role of plasmepsin II and III in piperaquine resistant P. falciparum lines
Bopp, Selina; *Summers, Robert; Walsh, Breanna; Volkman, Sarah; Wirth, Dyann*

101: Engineering a self-targeting entry inhibitor for vectored malaria prophylaxis
Srinivasan, Prakash

102: END-seq: a new whole-genome approach for the analysis of DNA double-stranded breaks in the African Trypanosome
Hovel-Miner, Galadriel; *Sciascia, Nicholas; Quinn, McKenzie*

103: Synthesis of Sterols and Sphingolipids Protect Leishmania against Membrane Perturbing Agents, Osmostress and Starvation
***Moitra, Samrat**; Xu, Wei; Zhang, Kai*

104: Novel uses of familiar reagents: A method for enrichment of ring stage Plasmodium falciparum
***Brown, Audrey C**.; Guler, Jennifer*

105: Optimized CRISPR/Cas9 protocols permit new gene editing applications in P. falciparum
***Crater, Anna**; Desai, Sanjay*

106: The Role Of Encephalitozoon Hellem Sporoplasm Protein 1 (Ehspp1) In Microporidia Development And Host Cell Invasion
***Han, Bing**; Weiss, Louis*

107: Malaria Parasites Require a Divergent Heme Oxygenase for Apicoplast Maintenance
***Nasamu, Sebastian**; Beck, Josh; Caaveiro, Jose; Niles, Jacquin; Goldberg, Daniel; Sigala, Paul*

108: TgCentrin2 regulates invasion and proliferation by the human parasite Toxoplasma gondii
***Leung, Jacqueline M**.; Liu, Jun; Wetzel, Laura; Murray, John M.; Hu, Ke*

109: Dissecting the role of RPA1 in the Leishmania major response to DNA replication stress
***Bastos, Matheus Silva**; Silva, Gabriel Lamak Almeida; Virgilio, Stela; Damasceno, Jeziel Dener; McCulloch, Richard; Tosi, Luiz Ricardo Orsini*

110: Characterization of the chromatin binding profile of RPA and 9-1-1 complexes of Leishmania major in response to replication stress
***Virgilio, Stela**; Bastos, Matheus Silva; Damasceno, Jeziel Dener; McCulloch, Richard; Tosi, Luiz Ricardo Orsini*

111: Investigating The Interactome Of The Toxoplasma Gondii Cyst Wall
***Tu, Vincent; Sugi**, Tatsuki; Tomita, Tadakimi; Yakubu, Rama; Han, Bing; Ma, Yanfen; Mayoral, Joshua; Williams, Tere; Weiss, Louis*

112: A kinetic assay provides mechanistic insights into malaria parasite protein trafficking and insertion at the host membrane

***Shao, Jinfeng; Saggu**, Gagandeep Singh; Desai, Sanjay*

113: Altered Morphology of the Digestive Vacuole Disrupts Integrity of the Endolysosomal System in Toxoplasma gondii
***Thornton, Brock**; Teehan, Paige; Cochrane, Christian; Floyd, Katherine; Bergmann, Amy; Dou, Zhicheng*

114: TIMP-1 promotes hypermigration of T. gondii-infected dendritic cells through CD63 / ITGB1 / FAK signaling
***Olafsson, Einar B**.; Barragan, Antonio*

115: Critical role of phosphorylation of the malarial cGMP-dependent protein kinase (PKG)
***Kousis, Konstantinos**; Withers-Martinez, Chrislaine; Baker, David A.; Blackman, Michael J.*

116: Plasmodium falciparum ubiquitin transferase, a novel putative quinine resistance marker
***Jankowska-Döllken, Monika**; Sanchez, Cecilia; Lanzer, Michael*

117: An apical motility switch in Toxoplasma gondii
***Brown, Kevin M**.; Sibley, L. David*

118: Genome-wide Screening of Copy Number Variation in Plasmodium falciparum Clinical Isolates
***Assisi, Christina**; Bozdech, Zbynek*

119: A molecular basis for the control of local Toxoplasma gondii in wild South American Mus musculus populations.
***Alvarez, Catalina**; Müller, Urs; Campos, Claudia; Steinfeldt, Tobias; Howard, Jonathan*

120: New Permeation Pathways in the host red cell during Plasmodium falciparum gametocytogenesis: greater than expected
***Bouyer, Guillaume**; Barbieri, Daniela; Marteau, Anthony; Roman, Diana; Dupuy, Florian; Sissoko, Abdoulaye; Siciliano, Giulia; Gomez, Lina; Pietro; Martins, Rafael; Lopez-Rubio, José-Juan; Clain, Jérome; Duval, Romain; Egée, Stéphane; Lavazec, Catherine*

121: A membrane associated PPM family protein phosphatase, PPM5C, regulates Toxoplasma gondii attachment to host cells
Yang, Chunlin

122: The apicomplexan membrane-trafficking system: gains, losses, and novel features
***Klinger, Christen M**.; Dacks, Joel B.; Meissner, Markus*

123: Push and Pull: the role of actin in invasion
***Del Rosario, Mario**; Latorre-Barragan, Fernanda; Das, Sujaan; Pall, Gurman; Stortz, Johannes Felix; Whitelaw, Jamie; Periz, Javier; Lemgruber, Leandro; Meissner, Markus*

124: Ribozyme-Mediated, Multiplex CRISPR Gene Editing and CRISPRi in Plasmodium
***Walker, Michael P**.; Singh, Suprita; Josling, Gabrielle; Llinás, Manuel; Lindner, Scott*

125: Rapid block of RNA splicing by chemical inhibition of analog-sensitive CRK9, a cyclin-dependent kinase essential in trypanosomes
***Gosavi, Ujwala A**.; Srivastava, Ankita; Gunzl, Arthur*

126: Analyzing the basic function of the positionally conserved PAP1 intron in Trypanosoma brucei
***Srivastava, Ankita**; O'Connor, Zachary; Gunzl, Arthur*

127: How is the Glideosome Associated Connector driving parasite motility?
***Dos Santos Pacheco**, Nicolas; Vadas, Oscar; Jacot, Damien; Tosetti, Nicolò; Han, Huijong; Kursula, Inari; Soldati-Favre, Dominique*

128: A novel lipid-binding protein mediates rhoptry discharge and invasion in Plasmodium falciparum and Toxoplasma gondii parasites
***Lebrun, Maryse**; Suarez, Catherine; Lentini, Gaelle; Maynadier, Marjorie; Aquilini, Eleonora; Ramaswamy, Raghavendran; Berry-Sterkers, Laurence; Cipriano, Michael; Chen, Alan; Bradley, Peter; Striepen, Boris; Boulanger, Martin*

129: Functional mapping of the ap2-g promoter in Plasmodium falciparum
***Basson, Travis**; Voss, Till S.*

130: Charting the Basis for T. gondii Virulence Traits Through Adaptive Laboratory Evolution (ALE)
***Primo, Vincent;** Farrell, Andrew; Rezvani, Yasaman; Vajdi, Amir; Marth, Gabor; Zarringhalam, Kourosh; Gubbels, Marc-Jan*

131: Identifying novel factors associated with trypanosome DNA replication forks using nascent DNA proteomics
***Klingbeil, Michele M**.; Rocha-Granados, Maria C.; Dodard, Garvin A.; Gunzl, Arthur*

132: Mobile Games: A Novel Instructional Tool to Teach Invasion of Red Blood Cells by Plasmodium falciparum
Comunale, Mary Ann*; Harvey, John; Vaidya, Akhil; Burns, Jim; Bergman, Lawrence; Wigdahl, Brian; Urdanta-Hartmann, Sandra*

133: Identification of Plasmodium intrinsic factors influencing gametocytes infectivity to Anopheles mosquitoes in the field
Ouologuem, Dinkorma*; Kone, Aminatou; Dara, Antoine; Diallo, Nouhoum; Dembele, Laurent; Ballo I., Fatoumata; Sangare, Cheick Oumar; Sangare, Boubou; Haidara, Aboubecrin; Dembele, Demba; Traore, Aliou; Doumbo, Ogobara K.; Djimde, Abdoulaye*

134: Antimalarial drug exposure triggers the formation of cytosolic hemozoin compartments in Plasmodium falciparum parasites
Maleki, Sharareh*; Rohrbach, Petra*

135: Comparative Genomics and Network Modeling of Parasites
Carey, Maureen *(University of Virginia); Stolarczyk, Michal (University of Virginia); Untariou, Ana (University of Virginia); Medlock, Gregory (University of Virginia); Guler, Jennifer (University of Virginia); Papin, Jason (University of Virginia)*

136: TgPL3 is a microtubule associated virulence factor with patatin-like phospholipase A2 and lipoxygenase activity
Wilson, Sarah*; Koch, Lindsey; Morrissette, Naomi; Knoll, Laura*

137: Small volume, cryopreserved Plasmodium vivax isolates for RNAseq studies via the SmartSeq2 platform
Rangel, Gabriel*; Clark, Martha; Goldberg, Jon; Kanjee, Usheer; Ferreira, Marcelo; Nusbaum, Chad; Neafsey, Daniel; Duraisingh, Manoj*

138: A parasite phosphorylation-ubiquitination axis licenses egress of Plasmodium falciparum from host erythrocytes
Paul, Aditya S.; *Paulo, Joao A.; Goldberg, Jon; Elsworth, Brendan; Kosber, Aziz L.; Gygi, Steven P.; Duraisingh, Manoj T.*

139: Investigating the Plasmodium epitranscriptome as a translational control mechanism
Sinha, Ameya*; Chee Sheng, Ng; Nah, Qianhui; Dedon, Peter; Preiser, Peter*

140: Disruption of the de novo heme biosynthesis pathway leads to severe defects of growth and acute virulence in Toxoplasma gondii

***Dou, Zhicheng**; Floyd, Katherine; Bergmann, Amy; Dameron, Carly*

141: Single Oral Dose Cethromycin Cures P. berghei Liver Stage Malaria Initiated by Mosquito Bites
***Sullivan, David J**.; Kennedy, Grace; Evans, Rachel; Poti, Kristin; Bobb, Bryce; Kaludov, Nick*

142: Loss of a deubiquitinase, TgOTUD5 delays cell cycle progression and breakdown of maternal remnants in Toxoplasma gondii.
***Dhara, Animesh**; Murphy, Robert; Sinai, Anthony P.*

143: The glucan phosphatase, TgLaforin, regulates amylopectin metabolism in both T. gondii tachyzoites and bradyzoites.
***Murphy, Robert D**.; Dhara, Animesh; Watts, Elizabeth; Brizzee, Corey; Sinai, Anthony; Gentry, Matthew*

144: Hemozoin in isogenic drug-resistant P. falciparum is smaller in size
Sayeed, Abeer

145: Rapid and specific drug-induced transcriptional responses to common antimalarials in asexual P. falciparum
***Painter, Heather**; Llinás, Manuel*

146: Cracking the coccidian egg: A molecular exploration of oocyst viability
***Kruth, Perryn**; R Barta, John*

147: eIF2a phosphorylation in Trypanosoma cruzi is required for generation of trypomastigotes from intracellular amastigotes
***Machado, Fabrício**; Malvezzi, Amaranta M.; Costa, Mirella; Schenkman, Sergio*

148: Trypanosoma brucei antigenic variation in extravascular spaces
***Mugnier, Monica**; Bobb, Bryce; Rijo-Ferreira, Filipa; Figueiredo, Luisa*

149: Proteomic analysis reveals that the association of VSPs and kinases with lipid rafts in Giardia is important for encystation and host-parasite interactions
***Grajeda, Brian**; De Chatterjee, Atasi; Pence, Breanna; Polanco, Gloria; Roychowdhury, Sukla; Almeida, Igor; Das, Siddhartha*

Poster Session C

Wednesday, September 12, 2018

3:00 pm – 5:00 pm

Poster Numbers 150 - 227

150: Mutations in the actin-binding protein PfCoronin confer resistance to Artemisinin in West African Plasmodium falciparum isolates
Sharma, Aabha

151: Mind the traffic: a role for trafficking in the cell stress response and artemisinin resistance in P. falciparum
Henrici, Ryan*; Zoltner, Martin; Hart, Melissa; Edwards, Rachel; van Schalkwyk, Don; Baker, David; Moon, Rob; Odom John, Audrey; Field, Mark; Sutherland, Colin*

152: Characterizing known translation inhibitors and drug candidates via Plasmodium falciparum whole cell extracts vs. S35 methionine incorporation: separating true 80S ribosome inhibition from artifact
Sheridan, Christine; *Garcia, Valentina; Ahyong, Vida; DeRisi, Joseph*

153: Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi
Berná, Luisa*; Rodriguez, Matias; Chiribao, Maria Laura; Parodi-Talice, Adriana; Pita, Sebastián; Rijo, Gastón; Alvarez-Valin, Fernando; Robello, Carlos*

154: Functional characterization of mitochondrial translation components in the early diverging eukaryote Toxoplasma gondii
Lacombe, Alice; *Tottey, J.; Ovciarikoval, J.; Courjol, F.; Gissot, M.; Sheiner, L.*

155: Antimalarial Drug Target PfATP4 is Present in Parasite Plasma Membrane as a Large Complex
Ramanathan, Aarti*; Vaidya, Akhil; Bhatnagar, Suyash; Morrisey, Joanne*

156: Transcription and localisation of sexual stage parasites in the P. chabaudi mouse malaria model.
Cunningham, Deirdre A*.; Deroost, Katrien; Hosking, Caroline; Manni, Sarah; Vandomme, Audrey; Lewis, Matthew; Langhorne, Jean*

157: Structure of a novel dimeric lysine methyltransferase that regulates the motility of the human parasite Toxoplasma gondii
Pivovarova, Yulia*; Liu, Jun; Lesigang, Johannes; Hu, Ke; Dong, Gang*

158: Analysis of the Toxoplasma F-box Protein 1, FBXO1, Reveals That During Endodyogeny the Daughter Cell Scaffold Forms Before Centrocone Duplication
Baptista, Carlos G*.; Lis, Agnieszka; Dittmar, Ashley; Deng, Bowen; West, Christopher; Blader, Ira*

159: A secondary metabolite produced by a mollusk symbiont has activity against multiple apicomplexan parasites.
Driskell, I*; O'Connor, RM; Beaushaw, J; Bowden, G; Lin, Z; Schmidt, E; Schafer, D; Riggs, M; Gimenez, F; Allred, D*

160: Strand-specific RNA Sequencing in Zoonotic Protozoan Pathogen Cryptosporidium parvum Suggests Widespread and Developmentally Regulated Long Non-coding RNA Transcription
Li, Yiran*; Baptista, Rodrigo; Sateriale, Adam; Striepen, Boris; Kissinger, Jessica C*

161: Molecular dissection of the Plasmodium protease plasmepsin V
Polino, Alexander; *Goldberg, Daniel*

162: Phenotypic changes in Toxoplasma gondii when the Spindly O-fucosyltransferase is knocked out
Samuelson, John*; Bandini, Giulia; Ichikawa, Travis; van der Wel, Hanke; Haserick, John; Costello, Catherine; West, Christopher*

163: A genetic approach for understanding how malaria parasites correlate DNA replication and cytokinesis during the blood-stage life cycle.
Absalon, Sabrina*; Dvorin, Jeffrey D*

164: Toxoplasma Rop16III facilitates cyst development through host cell manipulations.
Kochanowsky, Joshua*; Koshy, Anita*

165: A Proteomic Approach Reveals the Molecular Manipulation of the Stress-Response Pathway in Trypanosoma brucei Induced by Down Regulation of the Mitochondrial Protein Translocase, Tim50
Chaudhuri, Minu*; Singha, Ujjal; Tripathi, Anuj; Chaudhuri, Minu; Rose, Kristie; Sakhare, Shruti; Pratap, Siddharth*

166: Identification of a Novel Protein Phosphatase 1 Complex Involved in RNA Polymerase II Transcription Termination in Kinetoplastids
Kieft, Rudo*; zhang, yang; moran, jose; sabatini, Robert*

167: Investigating ApiAP2 proteins with similar DNA binding specificities in Plasmodium falciparum
Bonnell, Victoria A*.; Josling, Gabrielle; Russell, Timothy; Painter, Heather; Llinás, Manuel*

168: Quantification of amino acid metabolism and protein synthesis in different strains of the apicomplexan parasite, Toxoplasma gondii
Monahan, Colleen*; Salladay, Ivan; Pace, Douglas*

169: Identifying protein-protein interactions for the P. falciparum Merozoite Organizing Protein
McGee, James P.; *Absalon, Sabrina; Rudlaff, Rachel M.; Dvorin, Jeffrey D.*

170: Role of Toxoplasma OTU-family deubiquitinases in the selection of cell cycle architecture and developmental transitions.
Sinai, Anthony P*.; Dhara, Animesh; Lynn, Bert*

171: Analyzing the function of branched-chain alpha-keto acid dehydrogenase (BCKDH) in Plasmodium falciparum
Munro, Justin T*.; Allman, Erik; Llinás, Manuel*

172: Molecular detection & isolation of Benzimidazole resistant Haemonchus contortus and evaluation of Anthelmintic activity of Herbal formulation
Qamar, Muhammad Fiaz MFQ*; Ali, Kazim; Muhammad Arfan, Zaman; Muhammad, Younus; Ihtasham, Khan; Ehtisham ul, haq; Rabia, Tamkeen; Muhammad Imran, Rashid*

173: Erythrocyte Calcium-ATPase Activity Measured with Two Methods: Towards Novel Inhibitors as Antimalarials
Sims, Jeremiah N*.; Desai, Sanjay*

174: Comparative Transcriptomics in L. braziliensis: disclosing differential gene expression of coding and putative non-coding RNAs throughout developmental stages
Teles, Natália*; Ruy, Patrícia; Magalhães, Rubens; Dias, Leandro; Castro, Felipe; Myler, Peter; Cruz, Angela*

175: Population Genetics of Plasmodium falciparum and Plasmodium vivax in Islands of Vanuatu over a Decade
Dowd, Simone*; Gray, Karen-Ann; Kaneko, Akira; Auliff, Alyson; Taleo, George; Vastergaard, Lasse; Auburn, Sarah; Cheng, Qin*

176: Identifying host components of the membrane enveloping intracellular Toxoplasma gondii
Cygan, Alicja; Branon, *Tess; Ting, Alice; Boothroyd, John*

177: Compiling a minicircle genome of Trypanosoma brucei
Yu, Tian*; Zhang, Liye; Monti, Stefano; Aphasizhev, Ruslan*

178: Development of a new optogenetic tool to study essential genes in Cryptosporidium parvum
Vinayak, Sumiti*; Rose, Savannah; Gartlin, Brina*

179: Characterization the functional role of the Plasmodium falciparum AP2-G2 in gametocyotogenesis
Singh, Suprita*; Santos, Joana; Josling, Gabrielle; Orchard, Lindsey; Painter, Heather; Llinas, Manuel*
180: The Protein-Protein Interaction Landscape of the Apicomplexan Parasite Toxoplasma gondii
Stevens, Grant C*.; Swapna, Lakshmipuram; Brand, Verena; Boyle, John; Grigg, Michael; Emili, Andrew; Parkinson, John*

181: Use of micro RNA (mi RNA) therapy against Toxocara vitulorum in buffalo calves
Ayaz, Muhammad Mazhar

182: Biogenesis of Giardia intestinalis mitosomes
Voleman, Lubos*; Tumová, Pavla; Doležal, Pavel*

183: Inducing massive sexual conversion in Plasmodium falciparum with a conditional pfap2-g over-expression system
Llorà-Batlle, Oriol*; Michel-Todó, Lucas; Cortés, Alfred*

184: Challenging the importance of Pantothenate and Coenzyme A biosynthesis during acute and chronic stages of Toxoplasma infection
Lunghi, Matteo*; Krishnan, Aarti; Soldati-Favre, Dominique*

185: New insights in the pir genes expression in Plasmodium chabaudi : a first step to decipher their function.
Vandomme, Audrey*; Talavera-López, Carlos; Brugat, Thibaut; Amis, Sarah; Hosking, Caroline; Cunningham, Deirdre; Langhorne, Jean*

186: Intricate hierarchical transcriptional control regulates Plasmodium falciparum sexual differentiation and maturation
Van Biljon, Riette*; van Wyk, Roelof; Orchard, Lindsey; Painter, Heather; Reader, Janette; Niemand, Jandeli; Llinás, Manuel; Birkholtz, Lyn-Marie*

187: Impact of haemoglobin S on Plasmodium falciparum infected erythrocytes immune recognition in pregnancy-associated malaria
Chauvet, Margaux; *Tetard, Marilou; Cottrell, Gilles; Lohezic, Murielle; Pineau, Damien; Denoyel, Luc; Aussenac, Florentin; Roman, Jocelyne; Brossier, Emeline; Hanny, Marion; Luty, Adrian J.F.; Gamain, Benoît; Merckx, Anaïs; Migot-Nabias, Florence*

188: Comparative mapping of Plasmodium proteomes provides new insights into erythrocyte remodeling
Preiser, Peter*; Siau, Anthony; Hoo, Regina; Sheriff, Omar; Tay, Donald; Yam, Xue Yan; Loh, Han Ping; Mutwil, Marek; Wei, Meng; Sze, Siu Kwan*

189: Characterisation of an ApiAP2 factor associated with heterochromatin
Carrington, Eilidh*; Keller, Dominique; Toenhake, Christa G.; Bartfai, Richard; Voss, Till S.*

190: Screening Plasmodium falciparum sexual commitment
Brancucci, Nicolas M. B*.; Hitz, Eva; Voss, Till S.*

191: The dynamics of DNA replication during male gamete formation in Plasmodium falciparum
Matthews, Holly*; Merrick, Catherine*

192: Signal peptide recognition particle-based protein targeting in mitochondria of Naegleria gruberi
Pyrih, Jan*; Cimrhanzlová, Kristýna; Rašková, Vendula; Kriegová, Eva; Pánek, Tomáš; Tsaousis, Anastasios; Eliáš, Marek; Lukeš, Julius*

193: Chemical Proteomic Identification and Characterization of the Druggable Thiolome in Toxoplasma gondii
Child, Matthew*; Benns, Henry; Bogyo, Matthew; Weerapana, Eranthie; Tate, Edward*

194: New way to treat malaria based on cell biology
Wahlgren, Mats*; Leitgeb, Anna; Moll, Kirsten; Dondorp, Arjen*

195: Investigating the essential nature of a citrate synthase-like protein in Plasmodium falciparum
Nicklas, Sezin K*.; Mather, Michael; Ke, Hangjun; Vaidya, Akhil*

196: Antibodies to Plasmodium falciparum glutamic acid rich protein (PfGARP) protect against infection and severe disease
Raj, Dipak; *Dasmohapatra, Alok; Janiwali, Anup; Kurtis, Jonathan; Duffy, Patrick; Fried, Michal; Friedman, Jennifer*

197: Targeting a novel drug resistance pathway in P. falciparum
Fagbami, Lola

198: Role of the RNA binding protein RBP42 in Trypanosoma brucei bloodstream form parasites
Das, Anish; *Bellofatto, Vivian*

199: Assessment of the sensitivity and accuracy of the malaria Taqman array card in a field setting
Guler, Jennifer L*.; Kelly, Gillean; Dwomoh, Emmanuel; Warthan, Michelle; Pholwat, Suporn; Nsobya, Samuel; Mwanga, Juliet; Rosenthal, Phillip; Houpt, Eric; Kassaza, Kennedy*

200 Parasite specific labeling of N-acetylgalactosamine using a simple and robust ester-esterase pair system
Tomita, Tadakimi*; Wu, Peng; Weiss, Louis*

201: Creation of a tissue culture model for Toxoplasma gondii sexual development
Di Genova, Bruno; *Wilson, Sarah; Spence, Jason; Dubey, Jitender; Knoll, Laura*

202: Elucidating the mechanism of mitochondrial fission in Plasmodium falciparum
Mulaka, Maruthi*; Dass, Swati; Ke, Hangjun*

203: Probing the organization and function of the Plasmodium falciparum parasitophorous vacuole membrane
Beck, Josh*; Garten, Matthias; Heuser, John; Nessel, Timothy; Roth, Robyn; Zimmerberg, Joshua; Goldberg, Daniel*

204: Identification and targeting of male gamete specific factors involved in sexual reproduction during Cryptosporidium infection
English, Elizabeth*; Sateriale, Adam; Beiting, Daniel; Striepen, Boris*

205: **C-Type Cytochrome Maturation and Function in Malaria Parasites *Espino, Tanya****; Marvin, Rebecca; Nalder, Shai-anne; Sigala, Paul*

206: Imidazo[1,2b]pyridazines targeted against Plasmodium falciparum malaria block production of phosphatidylinositol-3-phosphate.
Safeukui, Innocent*; Liu, Rui; Pandharkar, Trupti; Bhattacharjee, Souvik; Liu, Haining; Estiu, Guillermina; Shirey, Carolyn; Stahelin, Robert; Mader, Mary; Zink, Richard; Margolis, Brandon; Ochoada, Jason; Montrose-Rafizadeh, Chahrzad; Duffy, Sandra; Avery, Vicky; Burrows, Jeremy; Bathrust, Ian; Wiest, Olaf; Grese, Timothy; Miller, Marvin; Haldar, Kasturi*

207: RTP4 is a negative regulator of IFN-I response during Plasmodium yoelii infection
He, Xiao*; Wu, Jian; Xia, Lu; Peng, Yu-chih; Tumas, Keyla; Liu, Chengyu; Myers, Timothy G.; Su, Xin-zhuan*

208: MitoNEETS: mitochondrial redox sensitive iron sulfur cluster transfer proteins in malaria parasites
Dass, Swati*; Mulaka, Maruthi; Morrisey, Joanne; Mather, Michael; Ke, Hangjun*

209: Characterizing novel Fis1 interactors to examine mitochondrial dynamics in Toxoplasma gondii
Jacobs, Kylie*; Charvat, Robert; Garbuz, Tamila; Arrizabalaga, Gustavo*

210: Optimization of an approach to detect copy number variation in a single parasite genome
Liu, Shiwei*; Guler, Jennifer*

211: Pyruvate Kinase II is Required for Apicoplast Maintenance
Swift, Russell*; Keutcha, Cyrianne; Liu, Hans; Prigge, Sean*

212: Reticulocyte conditioned media stimulates gametocytogenesis in Plasmodium falciparum culture
Evans, Rachel M.; *Sullivan, David; Wu, Mengyao; Cheng, Linzhao; Gao, Yongxing*

213: Stochastic Gene Expression in Plasmodium falciparum Asexual Stages: A Single Cell Transcriptomics Approach
Tripathi, Jaishree*; Zhu, Lei; Bozdech, Zbynek*

214: Functional characterization of a novel P. falciparum serine hydrolase important for ring stage formation and early intraerythrocytic development.
Ridewood, Sophie*; Annett, Dara; Deu, Edgar*

215: ER resident PfGRP170 is an essential protein in the human malaria parasite, Plasmodium falciparum
Kudyba, Heather M.; *Cobb, David W.; Florentin, Anat; Fierro, Manuel; Rodriguez, José; Ravishankar, Rajani; Muralidharan, Vasant*

216: An ER-resident Hsp40 is required for the asexual development of the malaria parasite P. falciparum.
Cobb, David W.; *Kudyba, Heather M.; Bruton, Baylee; Muralidharan, Vasant*

217: Epigenetic reader complexes of Plasmodium falciparum
Hoeijmakers, Wieteke; *Bartfai, Richard; Miao, Jun; Schmidt, Sabine; Shrestha, Sony; Venhuizen, Jeron; Henderson, Rob; Birnbaum, Jakob; Ghidelli, Sonja; Drewes, Gerard; Cui, Liwang; Stunnenberg, Hendrik; Spielmann, Tobias*

218: Post-translational Processing of a Secreted Putative Phosphatase in Toxoplasma gondii
Blakely, William

219: An ER-resident calcium binding protein is required for egress and invasion of malaria parasites
Fierro, Manuel A.; *Asady, Beejan; Brooks, Carrie; Moreno, Silvia; Muralidharan, Vasant*

220: Adaptation of Translation-Associated Machinery to the polyA Track-Rich Transcriptome in Plasmodium falciparum
Erath, Jessey; *Pavlovic-Djuranovic, Slavica; Djuranovic, Sergej*

221: H+-dependent inorganic phosphate uptake in Trypanosoma brucei is influenced by myo-inositol transporter
Heise, Norton; *Russo-Abrahão, Thais; Koeller, Carolina; Silva-Rito, Stephanie; Steinmann, Michael; Marins-Lucena, Thaissa; Alves-Bezerra, Michele; Lima-Giarola, Naíra; Francisco de-Paula, Iron; Gonzalez-Salgado, Amaia; Sigel, Erwin; Bütikofer, Peter; Calp Gondim, Kátia; Meyer-Fernandes, José Roberto*

222: Pharmacological disruption of ApiAP2 transcription factor function
Russell, Timothy J.; *DeSilva, Erandi; Bath, Jade; Josling, Gabrielle; Painter, Heather; Panagiotou, Gianni; Kirsch, Sierra; Fiddock, David; Llinás, Manuel*

223: Post-transcriptional regulation of the NT3 purine transporter in the Leishmania donovani purine stress response
Licon, Haley; *David, Larry; Landfear, Scott; Yates, Phillip*

224: CCCH zinc finger proteins regulate bradyzoite transition in Toxoplasma gondii.
***Garfoot, Andrew**; Knoll, Laura*

225: Investigating mutational pathways to resistance for clinically-relevant dihydroorotate dehydrogenase inhibitors
***Mandt, Rebecca**; Lafuente-Monasterio, Maria Jose; Sakata-Kato, Tomoyo; Luth, Madeline; Segura, Delfina; Pablos, Alba; Ottilie, Sabine; Winzeler, Elizabeth; Gamo, F. Javier; Wirth, Dyann; Lukens, Amanda*

226: Externalized neutrophil components drive inflammatory pathogenesis in malaria
***Knackstedt, Lorenz**; Mordmuller, Benjamin; Abu-Abed, Ulrike; Zychlinsky, Arturo; Amulic, Borko*

227: Splicing, translation and mRNA decay in Trypanosoma brucei
***Clayton, Christine (ZMBH**); Bajak, Kathrin (ZMBH); Bishola, Tania (ZMBH); Helbig, Claudia (ZMBH); Liu, Bin (ZMBH); Melo do Nascimento, Larissa (ZMBH); Terrao, Monica (ZMBH); Waithaka, Albina (ZMBH)*

Session I: Seeing is Believing

Chairs: Matthew Child and Veronica Jimenez

Sunday, September 9, 2018

7:00 pm – 9:00 pm

FT1: CryoEM Reveals Translocation Mechanism in Malaria Parasite Effector Export

Ho, Chi-Min (UCLA Molecular Biology Institute)*; Beck, Josh R. (Washington University in St. Louis, Iowas State University); Lai, Mason (University of California, Los Angeles); Cui, Yanxiang (University of California, Los Angeles); Goldberg, Daniel E. (Washington University in St. Louis); Egea, Pascal F. (University of California, Los Angeles); Zhou, Z. Hong (University of California, Los Angeles)*

The putative Plasmodium Translocon of Exported Proteins (PTEX) is essential for transport of malarial effector proteins across a parasite-encasing vacuolar membrane into host erythrocytes, but without structural information, the mechanism of this membrane protein complex in effector export remains unknown. Here we show PTEX is a bona fide translocon by determining near-atomic resolution cryoEM structures of the endogenous PTEX core complex of EXP2, PTEX150 and HSP101, isolated from Plasmodium falciparum in the engaged and resetting states of endogenous cargo translocation with the aid of CRISPR/Cas9-engineered epitope tags. Our work reveals the mechanism of P. falciparum effector protein export and enables structure-based design of drugs targeting this unique translocon.

FT2: Flagellar cAMP signaling directs trypanosome navigation through the tsetse fly vector

Millius, Sebastian (Institute of Cell Biology, University of Bern, Bern, Switzerland); *DeMarco, Stephanie F. (Molecular Biology Institute, University of California, Los Angeles, USA); Rehmann, Ruth (Institute of Cell Biology, University of Bern, Bern, Switzerland); Wenzler, Tanja (Institute of Cell Biology, University of Bern, Bern, Switzerland); Florini, Francesca (Institute of Cell Biology, University of Bern, Bern, Switzerland); Roditi, Isabel (Institute of Cell Biology, University of Bern, Bern, Switzerland); Hill, Kent L. (Molecular Biology Institute, University of California, Los Angeles, USA)*

Social motility (SoMo) describes the coordinated migration of early procyclic (midgut form) trypanosomes on a semi-solid surface. It is known that knockdown or inhibition of the phosphodiesterase PDEB1 prevents SoMo and leads to an increase in intracellular cAMP, but its biological relevance has been disputed. By creating a deletion mutant in a tsetse fly-infectious strain of Trypanosoma brucei, and performing live microscopy, we show that PDEB1 is required for a defined step of the transmission cycle, the migration from the midgut lumen to the ectoperitrophic space via the peritrophic matrix. Without PDEB1, most parasites remain trapped in the lumen, unable to progress further to the proventriculus. These results show that cAMP signaling is crucial for successful transmission and are consistent with SoMo reflecting a specific part of the T. brucei life cycle. cAMP signaling might also operate in the mammalian host when trypanosomes leave the bloodstream and infiltrate other tissues such as the central nervous system.

FT3: Plasmodium falciparum displays heterogeneous binding patterns in engineered 3D brain microvessels

Bernabeu, Maria (Center for Infectious Disease Research, Seattle, WA)*; Gunnarsson, Celina (Department of Bioengineering, University of Washington, Seattle, WA); Vishnyakova, Maria (Center for Infectious Disease Research, Seattle, WA); Avril, Marion (Center for Infectious Disease Research, Seattle, WA); Nagao, Ryan J. (Department of Bioengineering, University of Washington, Seattle, WA); Taylor, Terrie E. (Department of Osteopathic Medical Specialties, College of Osteopathic Medicine, Michigan State University, MI); Seydel, Karl B. (Department of Osteopathic Medical Specialties, College of Osteopathic Medicine, Michigan State University, MI); Zheng, Ying (Department of Bioengineering, University of Washington, Seattle, WA); Smith, Joseph D. (Center for Infectious Disease Research, Seattle, WA)*

Cerebral malaria is a severe neurological complication of Plasmodium falciparum infection characterized by sequestration of infected erythrocytes (IE) in the brain microvasculature, mediated by the var/PfEMP1 family. Expression of group A and DC8 var genes is associated with severe malaria, but the specific host receptors responsible for cerebral sequestration remain under debate. A major obstacle towards understanding the mechanism of parasite sequestration is the inaccessibility of the brain and the lack of a suitable experimental animal model or in vitro brain endothelial model. Here, we have generated a 3D human brain microvessel model to study P. falciparum–brain endothelium interactions. Primary human brain endothelial cells in our model present brain endothelial characteristics, and establish adherens junctions, as revealed by VE-chadherin staining. Under controlled flow rates, our model displays a large gradient of physiological flow velocities and wall shear stress (WSS) that reproduces the brain vasculature in health and disease. We show that both EPCR and ICAM-1 contribute to the parasite-brain vessel interaction. Moreover, P. falciparum lines expressing different var/PfEMP1 variants implicated in severe malaria present a heterogeneous binding behavior that is WSS-dependent and differentially responsive to endothelial activation. Our results identify parasite binding interactions in 3D brain microvessels, and provide evidence for a bet-hedging strategy that allows the parasite to quickly adapt to a changing vascular environment during infection.

FT4: Cellulose, chitin, and three families of abundant proteins localize to distinct structures in the cyst wall of the eye pathogen Acanthamoeba castellanii

Magistrado-Coxen, Pamela (Boston University); *Aqeel, Yousuf; Lopez, Angelo; Haserick, John; Costello, Catherine; Samuelson, John*

Acanthamoeba castellanii trophozoites, cause of keratitis and blindness, form antibiotic-resistant cysts when nutrient-deprived. Acanthamoeba keratitis is an emerging disease, as the incidence and severity of infection is increased with use of contact lenses. Studies of cyst wall biology will contribute to our understanding of parasite transmission and may identify new targets for diagnostics and therapeutics. Unlike all other protist walls described to date, A. castellanii cyst walls contain both cellulose and chitin and have distinct inner (endocyst), outer (ectocyst) layers and conical structures (ostioles) attaching the layers. We used structured illumination microscopy (SIM) to show that cellulose and chitin are made in encystation-specific vesicles rather than at the plasma membrane, as is the case in plants and fungi, respectively. Cellulose is enriched in the ectocyst layer, while chitin is enriched in the endocyst layer and ostioles. We used mass spectrometry to show that A. castellanii cyst walls contain three encystation-specific families of abundant proteins. Representative members of each family tagged with EGFP and expressed under their own promoter in transfected parasites, localize to distinct structures in the cyst wall. CAA proteins, which contain one or three “choice of anchor” domains, are predominantly in the ectocyst layer. In contrast, Leo lectins, which contain two unique 8-Cys domains, and Luke lectins, which contain two or three carbohydrate-binding modules 49 first identified at the C-terminus of a tomato cellulose, are both enriched in the endocyst layer and ostioles. Presently we are using recombinant proteins to determine the carbohydrate-binding specificity of representative cyst wall proteins and using SIM to determine how cellulose, chitin, and proteins assemble into the distinct structures in the cyst wall.

FT5: Stem cell-derived epithelial culture system that supports complete Cryptosporidium parvum development in vitro

Funkhouser-Jones, Lisa J. (Washington University School of Medicine)*; Wilke, Georgia (Washington University School of Medicine); Ravindran, Soumya (Washington University School of Medicine); Wang, Yi (Washington University School of Medicine); Kuhlenschmidt, Mark (University of Illinois, Urbana-Champaign); Stappenbeck, Thaddeus (Washington University School of Medicine); Sibley, L. David (Washington University School of Medicine)*

The protozoan parasite Cryptosporidium parvum is a leading cause of pediatric diarrheal disease in developing countries, yet our understanding of its underlying biology and the development of effective therapeutics have been hindered by a lack of reliable in vitro culture systems that support continuous growth of the parasite. C. parvum infects and replicates through 2 – 3 cycles of asexual development in human adenocarcinoma cell lines but does not progress beyond the gamont stage to fertilization and oocyst formation. As such, these culture systems are short-lived and do not recapitulate the entire C. parvum life cycle. To overcome these limitations, we developed an improved culture system for C. parvum that uses primary mouse intestinal epithelial cell (mIEC) monolayers grown on transwells to better mimic the natural niche of the parasite. mIEC monolayers undergo differentiation to generate all subtypes of cells normally found in the intestinal epithelium, and continued proliferation of stem cells in the system supports long-term cultivation of differentiated monolayers. mIECs grown on transwells support robust growth of C. parvum for at least 3 weeks post infection, during which the parasite undergoes multiple rounds of replication. Unlike in adenocarcinoma cells, the C. parvum life cycle progresses through fertilization, meiosis, and oocyst maturation, as indicated by EdU incorporation in nascent sporozoites. Cultivation in mIECs supports generation of transgenic C. parvum lines in vitro, which can readily be amplified in immunocompromised mice via oral gavage. This system is being used to explore the processes of meiosis in vitro, the mechanism of action of anti-Cryptosporidium drugs, and tripartite interactions between host, parasite and microbiota in a tractable culture system.

TT1: Identification of the Plasmodium falciparum var gene interactome with CRISPR-ChIP

Bryant, Jessica M. (Institut Pasteur)*; Baumgarten, Sebastian (Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris); SCHERF, Artur (Institut Pasteur)*

Epigenetic control of mutually exclusive transcription within the ~60 member var gene family is important for pathogenesis of the malaria parasite Plasmodium falciparum. DNA elements shared by all var genes – a 5' upstream promoter region and a conserved intron – have been shown to play a role in modulating mutually exclusive transcription of var genes. However, only a few trans-acting factors have been implicated in this process. To investigate how a single var gene is targeted for transcription while all others are kept in a transcriptionally silent state, we attempted to identify new proteins and/or RNAs that bind to putative transcription control regions of var genes. We developed a multi-locus, CRISPR-mediated chromatin immunoprecipitation technology to isolate var gene loci in their natural chromatin environment in vivo. Using a tagged, catalytically inactive Cas9 ("dead" or dCas9), we targeted ~30% of var promoters or introns with a single guide RNA specific for each region. After crosslinking, immunoprecipitation of dCas9 allowed for co-purification of all proteins, DNA, and RNA closely associated with the targeted genomic DNA region. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) revealed that dCas9 binding was highly efficient and specific, with an average 500-fold enrichment of target loci and no off-target effects. High coverage identification of dCas9 by protein mass-spectrometry further confirmed the robustness of the method. Ongoing immunoprecipitation experiments, coupled with mass spectrometry and RNA sequencing, will identify new and interesting factors directly associating with var genes. This study provides a new tool for the unbiased and in vivo characterization of locus-specific cis- and trans-regulatory elements in one of the major virulence gene families of the malaria parasite.

TT2: Using humanized malaria parasites to study protein interactions at the erythrocyte cytoskeleton

Warncke, Jan (Swiss Tropical and Public Health Institute, Basel, Switzerland); *Beilstein, Sabina; Wyss, Matthias; Gabel, Anke; Passecker, Armin; Perez, Lara; Butter, Falk; Beck, Hans-Peter*

The extensive host cell remodelling of human erythrocytes during the course of Plasmodium falciparum infection is facilitated by a large number of exported parasite proteins. The function of the majority of these proteins remains elusive but we showed by immunoprecipitation that erythrocyte cytoskeleton proteins are potential interaction partners for many exported parasite proteins. To confirm these interactions reverse precipitations would be needed which is a major challenge due to the terminal differentiation and lack of nucleus in the erythrocyte. The interconnectivity of the cytoskeleton and its sticky nature requiring harsh lysis conditions adds an additional problem. To circumvent this holdback and to facilitate immunoprecipitations, we constructed parasite lines which express and export different tagged human cytoskeleton proteins. These human proteins were designed to be soluble within the cytosol of the infected erythrocyte and are expected to bind their putative parasite binding partners which are being transported to their final destination within the host cell. With this approach we can use these human proteins for immunoprecipitations and potentially modify human proteins within the infected cell. Here we present the expression and export of these proteins and show that several different N-terminal export signals could be used successfully and targeted the human protein for correct export into the host cytosol. We also present first MS data obtained using these humanized parasites demonstrating the usefulness of this system. This new humanized parasite approach offers immense potential for studying interactions between human cytoskeleton and exported parasite proteins as well as it allows manipulation of human cytoskeleton proteins to understand their role during the erythrocyte cycle of P. falciparum.

TT3: In vivo and in vitro studies of the host-parasite interactions of Spironucleus salmonicida and the Atlantic salmon

Ástvaldsson, Ásgeir; *Stairs, Courtney; Alfjorden, Anders; Hultenby, Kjell; Jansson, Eva; Svärd, Staffan*

The diplomonads are a diverse group of flagellated eukaryotic microbes adapted to life in oxygen depleted environments. An understudied member of the diplomonads, Spironucleus salmonicida, is known to be able to cause a systemic infection in salmonids (e.g. Salmo salar), resulting in economical losses in farmed fish during outbreaks of the parasite. Little is known about its life cycle and research is needed to give the ability to control for transmission and outbreaks. To shed a light on the life cycle we have infected juvenile Atlantic salmons with S. salmonicida and followed the course of infection up to 10 weeks in 5 individual trials. During these trials we have collected infected fish tissues for histological analyses and RNA sequencing. During two of the trials we intubated salmons with transfected parasites stably expressing the Firefly luciferase reporter, upon genomic integration, to monitor the infection closely using a Xenogen Ivis camera system. To dissect the host-parasites interaction further, we used an in vitro infection model including the Atlantic salmon kidney cell line (ASK) in a time course experiment for 24 hrs. RNA samples were collected at 6 different time points and we make an attempt to identify important transcriptional changes associated with the early stages of infection. Additionally, we have collected SEM and TEM images during the course of the experiment. These are the first molecular studies to look at the host-parasite interaction of S. salmonicida and salmon and the results give highly valuable information on the parasites behavior and are vital in the completion of the life cycle of S. salmoncidia.

TT4: Data mining and analysis using EuPathDB resources

Harb, Omar S. *Warrenfeltz, Suzzane; Brunk, Brian; Kissinger, Jessica; Roos, David*

The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource that facilitates data mining across a wide variety of datatypes. Pre-analyzed omics data can be interrogated through an advanced search strategy system and results can be visualized or analyzed further. In addition, primary data maybe be analyzed using a private Galaxy workspace where all EuPathDB genomes have been pre-loaded. EuPathDB supports over 230 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida, oomycetes and fungi. For these organisms, EuPathDB integrates a wide range of data including genome sequence and annotation, transcriptomics, proteomics, phenomics, epigenomics, metabolomics, population resequencing clinical and field isolates and host-pathogen interaction data. Data are analyzed using standard workflows and an in-house genome analysis pipeline generates data including domain predictions, orthology profiles across all genomes and GO term associations. Our unique strategies system offers over 100 structured searches that query the pre-computed data. Individual search results can be combined into strategies that easily merge evidence from diverse data types and across organisms. Easily accessible tools enhance the search strategy system and include dynamic data visualization, comparative genome analysis, population genetics tools, and functional or pathway enrichment. This comprehensive resource empowers the scientific community to mine data and develop hypothesis driven research. To learn more about what is new, ask us questions, contribute user comments or to provide suggestions visit our booth during all poster sessions.

Session II: “I have a gut feeling”: New Approaches for Intestinal Parasites

Chairs: Katherine Ralston and Josh Beck

Monday, September 10, 2018

9:00 am – 10:10 am

FT1: Genome-wide CRISPR/Cas9 Knockout Screen Identifies Host Genes Important for Cryptosporidium parvum Infection

Gibson, Alexis R. (University of Pennsylvania)*; Sateriale, Adam (University of Pennsylvania); Striepen, Boris (University of Pennsylvania)*

Cryptosporidium is an obligate intracellular protozoan parasite, and the second leading cause of severe diarrhea and diarrheal-related death in children worldwide. There are currently no vaccines and the only drug available has low efficacy in immunocompromised individuals who need it most. A lack of tools to study Cryptosporidium including continuous culture, molecular genetics, and immune competent animal models have impeded progress towards development of novel therapeutics. Due to these challenges, little is known about the host response to Cryptosporidium. We conducted a genome-wide CRISPR/Cas9 knockout screen to discover host genes necessary for Cryptosporidium parvum infection. Human intestinal epithelial cells, HCT-8s, stably expressing Cas9 were transfected with a lentiviral sgRNA library targeting every gene in the human genome. Selection for resistance to cell death was accomplished by three consecutive 72-hour C. parvum infections.

After each challenge, cells were removed for gDNA extraction and sequenced to determine the abundance of each sgRNA. The top 25 enriched genes after three replicates indicate that type III interferon signaling is important for susceptibility to C. parvum infection. Investigation into the role of type III interferon signaling in C. parvum infection will reveal how a viral defense pathway seems to promote parasite infection. Genes in pathways of host glycosaminoglycan synthesis and glycosylphosphatidylinositol (GPI) anchor synthesis were also amongst the top candidates, likely responsible for facilitating parasite attachment and invasion. Further research is necessary to understand the importance of these pathways in host defense and the role parasite manipulation may play in these responses.

FT2: Entamoeba histolytica trogocytosis contributes to acquisition of host cell membrane proteins and protection from lysis by human serum

Miller, Hannah W. (University of California, Davis); *Ralston, Katherine S. (University of California, Davis)*

Trogocytosis in E. histolytica is an actin-dependent endocytic process in which amoebae kill human cells by extracting and ingesting “bites” of human cell material. Trogocytosis is not exclusive to Entamoeba, but can be observed in other single-celled eukaryotic organisms as well as mammals. Mammalian immune cell trogocytosis results in transfer and display of plasma membrane proteins from the donor to the recipient cell. We aimed to determine if the transfer of human cell membrane proteins to amoebae occurred following trogocytosis of human Jurkat cells. Confocal microscopy and imaging flow cytometry revealed the transfer of biotinylated Jurkat membrane proteins as well as MHC-I molecules to the surface of amoebae via trogocytosis. Transfer of membrane proteins was quantitatively inhibited with cytochalasin D treatment. We next interrogated the hypothesis that acquisition and display of host cell membrane proteins allows E. histolytica to evade complement lysis. We found that amoebic ingestion of Jurkat cells resulted in protection from lysis by human serum. Protection was acquired from ingestion via trogocytosis of live human cells but not via phagocytosis of apoptotic human cells. Further supporting a role for trogocytosis, treatment of amoebae with cytochalasin D abrogated this protection, and preliminary transwell experiments show that protection requires contact with human cells. Collectively, these results suggest that amoebae acquire and display host membrane proteins through trogocytosis and that these proteins may confer protection against lysis by human complement. These studies have major implications for interactions between E. histolytica and the immune system, and further suggest that acquisition and display of membrane proteins is a general feature of trogocytosis that is not restricted to trogocytosis between mammalian immune cells.

FT3: Genetic evaluation of purine biosynthesis in Cryptosporidium parvum suggests nucleotide uptake from host cell

Pawlowic, Mattie C. (University of Dundee)*; Sateriale, Adam (University of Pennsylvania); Herbert, Gillian (University of Georgia); Matsuda, Rina (University of Pennsylvania); Gibson, Alexis (University of Pennsylvania); Mead, Jan (Emory University School of Medicine); Cuny, Gregory (University of Houston); Hedstrom, Lizbeth (Brandeis University); Striepen, Boris (University of Pennsylvania)*

The apicomplexan parasite Cryptosporidium is a leading global cause of severe diarrheal disease and an important contributor to early childhood mortality.
There is currently no fully effective treatment. Interference with nucleotide synthesis has been a mainstay of antibacterial and antiparasitic therapy and Cryptosporidium's highly reduced anabolic metabolism suggests multiple strong targets. Here we take advantage of newly available molecular genetics for Cryptosporidium to experimentally test the essentiality of multiple enzymes involved in nucleotide synthesis by gene ablation. Surprisingly, we found that the parasite tolerates the loss of classical targets including dihydrofolate reductase-thymidylate synthase (DHFR-TS) and inosine monophosphate dehydrogenase (IMPDH). We found that the parasite requires the ability to synthesize the pyrimidine nucleotide, thymidine monophosphate, but can rely on either thymidine kinase or DHFR-TS to do so. In contrast to the parasite's pyrimidine synthesis, the purine pathway appears to be non-redundant. Despite this, multiple enzymes in the purine pathway, including the adenosine transporter, can be ablated without measurable loss of fitness. The resulting mutants are viable under normal conditions but are hypersensitive to inhibition of host cell purine nucleotide synthesis. A combination of genetic and pharmacological experiments suggests that Cryptosporidium imports purines but not pyrimidines from the host cell. Although Cryptosporidium is clearly dependent on uptake of host-derived nucleotides, the specific substrates have yet to be determined. As Cryptosporidium lacks oxidative phosphorylation, it is possible that parasites balance that by uptake of ATP. A limited number of minimalist pathogens including Rickettsia, Chlamydia and Microsporidia import ATP and are considered energy parasites. The potential for ATP uptake from the host has significant impact to our understanding of Cryptosporidium metabolism and drug development.

FT4: An NAD+ dependent novel transcription factor controls stage conversion in Entamoeba

Manna, Dipak; *Lentz, Christian; Ehrenkauger, Gretchen; Suresh, Susmitha; Bhatt, Amrita; Singh, Upinder*

An NAD+ dependent novel transcription factor controls stage conversion in Entamoeba Dipak Manna1, Christian Lentz3#, Gretchen Ehrenkaufer1#, Susmitha Suresh1, Amrita Bhatt1 and Upinder Singh1,2 * 1Division of Infectious Diseases, Department of Internal Medicine, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA 2Department of Microbiology and Immunology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA 3Department of Pathology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA Abstract: Developmental switching between life-cycle stages is a common feature among parasitic pathogens to facilitate disease transmission and pathogenesis. The protozoan parasite Entamoeba switches between trophozoites and cysts, which are responsible for disease transmission. Despite being a central factor in amebic biology, stage inter-conversion is extremely poorly understood. We have identified a novel transcription factor ERM-BP (Encystation Regulatory Motif-Binding Protein) that regulates Entamoeba encystation by controlling stage specific gene expression. Down-regulation of ERM-BP decreases encystation efficiency and results in abnormal cysts with defective cyst walls. We determined that cellular NAD+ levels increase during encystation and exogenous NAD+ enhances encystation efficiency consistent with the known role of carbon source depletion in triggering Entamoeba encystation. We demonstrated that direct binding of NAD+ to ERM-BP affects ERM-BP conformation and facilitates promoter DNA binding. Additionally, we identified that ERM-BP mediates catalysis of nicotinamide to nicotinic acid, which may have second messenger effects to facilitate stage conversion. Our work links metabolic functions and NAD+ levels to transcriptional control of development in Entamoeba, thus providing the first insights into the molecular regulators of stage conversion.

TT1: Consistent, comparative and evidence-based genome annotation and re-annotation for the closely-related species, Cryptosporidium parvum, C. hominis and C. tyzzeri

Baptista, Rodrigo P. *Li, Yiran; Sateriale, Adam; Ansell, Brendan; Jex, Aaron; Cotton, James A.; Sanders, Mandy; Brooks, Karen; Tracey, Alan; Berriman, Matthew; Striepen, Boris; Kissinger, Jessica C.*

Genome sequences for the genus Cryptosporidium are currently being generated with regularity. However, because of issues with insufficient biological material for clinical isolates and the experimental resources needed for validation, fundamental gaps remain in the sequence assemblies and annotation. Currently, there are a few draft genome sequence assemblies for some Cryptosporidium species but they lack experimentally-supported genome annotation. In this work, we have generated a new C. parvum genome sequence assembly which lacks any gaps or ambiguous bases. It consists of 8 chromosomes and 13/16 telomeres are presente. Our goal here was to generate the best possible structural assembly and functional genome annotation for three closely-related species of Cryptosporidium, C. parvum IOWA, C. hominis 30976 and the new crypto mouse model C. tyzzeri. A manual curation of all genes in the context of existing molecular evidence and synteny was performed using a local installation of WebApollo2. In comparison to the previously available C. parvum IOWA annotation, > 1,500 annotation alterations were made. All genome sequences including the new C. tyzzeri genome sequence had a similar number of single-copy annotated genes, but some genes have become pseudogenes in one species or the other. These differences are likely the result of evolution but few are likely sequencing errors. The new functional analysis was also improved by adding motif, signal peptide, transporter and transmembrane information to some of the uncharacterized predicted proteins. These improvements allowed us to revisit the metabolic pathways of the parasite, including a new CryptoCYC database and look for choke points and potential new drugs targets based on orthology to known drug targets in public databases. Comparative approaches across these closely-related genome sequences facilitated the identification of both conserved and novel features, including copy number variation and a putative genome rearrangement. The new annotations are available at CryptoDB and GenBank for access by the research community. Additional experimental data are essential for bettering our understanding of Cryptosporidium.

TT2: Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to Giardia Intestinalis during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation

Ma'ayeh, Showgy Yasir; *Stadelmann, Britta; Knörr, Livia; Sköld, Karin; Svärd, Staffan*

Introduction: Giardia Intestinalisis a protozoan parasite that causes diarrhea in humans. Despite the manifestation of diarrheal disease, little inflammation is seen during infections indicating that Giardia might exert immunomodulatory responses in the host. Objective: Therefore, we aimed to study whether Giardia attenuates inflammation using an in vitro model of interaction. Method: We used transcriptomics to identify upregulated genes at early hours of interaction (1.5, 3 and 4h) between WB isolate and differentiated Caco2 cells (DCCs).

Results: A transcriptional peak at 1.5h was seen for genes encoding the cytokines/chemokines IL-8, CCL2, CCL20, CXCL1, CXCL2 and CXCL3 together with nuclear recruitment of proinflammatory genes transcription factors, nuclear factor kappa B (NFkB) and activator protein-1 (AP-1), declining afterwards. Despite the increased phosphorylation of Erk1/2 and P38, cytokines concentrations were low (< 200 pg/ml) and discordant with their transcriptional levels, indicating a post-transcriptional regulation of cytokine production. A gene encoding zinc finger protein 36 (i.e. tristetraprolin, TTP) was upregulated (1.5h) whose product is known to bind 3'UTR of cytokines transcripts inducing their decay. We show that Giardia induces TTP expression in DCCs and when incubated with inflamed DCCs the increase in TTP expression coincided with an inhibition of ERK1/2 and P38 phosphorylation. A reduction in Luciferase activity in DDCs transfected with a reporter plasmid with 3'UTR of IL8, CCL20 and CXCL1, confirmed the role of TTP in regulating cytokine production post-transcriptionally. Conclusion: This study highlights a role for Giardia-induced expression of anti-inflammatory factors in DCCs to control inflammation and enhance parasite persistence during infection.

Session III: Zombie: Taking Control of the Host

Chairs: Josh Beck and Katherine Ralston

Monday, September 10, 2018

10:30am – 11:30am

FT1: The First Steps Toward Dissecting the "Ins and Outs" of *T. cruzi* During Intracellular Infection

***Nathan Chasen*[1]**, *Rick L. Tarelton*[1] *and R. Drew Etheridge*[1]
[1]*Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA, 30602*

Trypanosoma cruzi, the causal agent of Chagas' disease, spends the majority of its time in the cytoplasm of mammalian host cells as actively replicating amastigotes, which convert into the highly motile and infectious trypomastigote form before escaping from the host-cell to further the infection. We know surprisingly little about how *T. cruzi* interacts with its host cell in terms of the mechanistic basis of metabolite uptake from the host cytoplasm or the potential release of parasite effectors that actively manipulate host-cell processes. We have, to our knowledge, identified the first resident protein (CP1) of the cytostome/cytopharynx complex, a long tubular invagination of the parasite membrane that is thought to be the primary site of amastigote and epimastigote endocytosis and thus an important route of nutritional uptake. We observed that endocytosis of BSA, Transferrin, and Concanavalin A all occurred along this tubular structure labeled by CP1. Our group has also demonstrated the first direct evidence of active secretion into the host-cell by intracellular *T. cruzi* by utilizing a host nuclear targeted fluorescent reporter construct (mNeon-Sec). Prior to secretion, mNeon-Sec accumulates in a compartment adjacent to the flagellar pocket of amastigotes, however upon differentiation into trypomastigotes, we observe a reduction of fluorescence within the transforming parasite and an increase in fluorescence of the host-cell nucleus; evidence of controlled release of the reporter into the host cytosol. The goal of this work is to generate tools that utilize CP1 and mNeon-Sec to elucidate the machinery and effectors involved in *T. cruzi* amastigote nutrient acquisition and host cell modulation.

FT2: The Exported FIKK Kinases of P. falciparum Target Key Proteins Involved in Host-cell Modification

Davies, Heledd M. (Francis Crick Institute)*; Treeck, Moritz (Francis Crick Institute); Belda, Hugo (Francis Crick Institute); Broncel, Goska (Francis Crick Institute); Tiburcio, Marta (Francis Crick Institute)*

Modification of the host erythrocyte by Plasmodium falciparum exported proteins is believed to be a key contributor to the severity of malaria symptoms caused by this parasite relative to other Plasmodium species. In addition to the formation of parasite-derived structures in the host cell such as adhesive knob structures and Maurer's clefts, infection by P. falciparum also leads to phosphorylation of several erythrocyte components. P. falciparum exports 20 members of the FIKK family of kinases into the host cell, while other human-infecting Plasmodium species possess only one non-exported FIKK kinase. We hypothesise that the FIKK kinases may therefore modulate the host cell modifications responsible for severe malaria. Using 10-plex quantitative phosphoproteomics we first analysed phosphorylation events that occur in the infected over the uninfected red blood cell and identified profound phosphorylation on RBC components upon infection.To elucidate the role of FIKKs in P. falciparum biology we conditionally inactivated each FIKK by flanking the kinase domain with loxP sites in a new, marker-free NF54::DiCre parasite line. For a cluster of FIKK genes on chromosome 9, an entire 25kb section was loxP-flanked in a single transfection step to allow the conditional deletion of 7 FIKKs simultaneously. We subsequently compared the phosphoproteome of each FIKK conditional knockout line to their WT counterparts. We observed strikingly different phosphorylation targets on both host and parasite proteins for different FIKKs suggesting their functions do not overlap. Additionally, recombinant expression of several FIKKs shows only partially overlapping substrate specificity. We are now using multiple phenotyping screens including electron microscopy, cytoadhesion assays and cell rigidity measurements to evaluate the contribution of each kinase to erythrocyte properties.

FT3: Systematic identification of Toxoplasma virulence factors in vivo

Sangare, Lamba Omar (University of California, Davis)*; Wang, Yifan (University of California, Davis); Krishnamurthy, Shruthi (University of California, Davis); Paredes-Santos, Tatiana C (University of California, Davis); Sidik, Saima M (Whitehead Institute for Biomedical Research, Cambridge); Lourido, Sebastian (Whitehead Institute for Biomedical Research, Cambridge); Saeij, Jeroen P.J (University of California, Davis)*

How eukaryotic parasites like Toxoplasma gondii modulate the host immune system, overcome in vivo nutrient deficiencies, and disseminates to distant organs is poorly understood. Toxoplasma co-opts the host cell by secreting proteins from specialized organelles called rhoptries (secreting ROPs) and dense granules (secreting GRAs). However, most ROPs and GRAs have unknown functions. We used CRISPR/Cas9 and a single guide RNA library designed to target 217 Toxoplasma GRA-encoding genes with which we created a pool of Toxoplasma loss-of-function (LOF) mutants, which we then used to infect mice and to study the effect of each gene on infectivity. This approach identified various genes that affected Toxoplasma fitness at the site of infection or dissemination and/or survival in distant organs. Because macrophages are one of the major cell types infected by Toxoplasma in vivo and because IFNγ is the major cytokine mediating resistance to Toxoplasma, we also performed genome-wide LOF screens in vitro to identify Toxoplasma genes required for fitness in IFNγ-stimulated and naïve macrophages. We further generated Toxoplasma single gene knockouts for some of our top hits, which were outcompeted by wild-type in in vitro and in vivo growth competition assays confirming the validity of the screens. For one of our hits, we confirmed an in vivo dissemination defect and identified that it interacts with the host WAVE regulatory complex, which is involved in the formation of the actin cytoskeleton through interaction with the Arp2/3 complex. We are currently investigating the hypothesis that this hit affects the dissemination of Toxoplasma by affecting migration of infected cells to distant sites.

TT1: Targeting Cell Entry As Therapy for Leishmaniasis

Wetzel, Dawn M.; *Ullah, Imran; Kernen, Rebecca M.; Booshehri, Laela M.; Rhodes, Emma L.; Mamula, Emily T.; Niederstrasser, Hanspeter; Posner, Bruce A.*

Leishmania is an obligate intracellular parasite that must be internalized by phagocytic cells in order to evade the host immune system and cause disease.
The uptake of Leishmania by macrophages is thought to be primarily host cell-driven. Previous work in the Wetzel laboratory demonstrated that the Abl/Arg and Src host cell kinases are required for efficient cell entry by Leishmania and maximal disease in a mouse model of cutaneous leishmaniasis. We have now identified additional components of the host cell signaling pathways required for Leishmania uptake by macrophages and subsequent disease in mice. In addition, we have adapted our two-color immunofluorescence assay for parasite internalization to a high throughput format. In conjunction with the UT Southwestern Medical Center High Throughput Screening Core, we plan to identify additional novel small molecules that affect Leishmania uptake. We hope that by continuing to study the uptake of Leishmania by macrophages, we will not only be able to identify new host cell pathways required for parasite internalization, but also suggest novel therapeutics for leishmaniasis.

TT2: Cryptosporidium parvum exports proteins into the cytoplasm of the epithelial host cell

Dumaine, Jennifer E,; *Sateriale, Adam; Reddy, Amita; Striepen, Boris*

Infection with the protozoan parasite Cryptosporidium is a leading cause of diarrheal disease and child mortality worldwide. Cryptosporidiosis is typically self-limiting, but in the context of malnourishment or immunodeficiency the disease can be protracted and deadly. Currently, there are no vaccines and only a single drug of limited efficacy. Upon infection, Cryptosporidium travels to the small intestine, where it establishes an intracellular but extracytoplasmatic parasitophorous vacuole leading to dramatic remodeling of the cytoskeleton of the epithelial cell. We hypothesize that effector proteins exported into the host cell play critical roles in establishment and maintenance of infection by modulating interaction with host immunity. We assembled a prioritized list of candidate effectors based on a variety functional genomic and population genetic parameters. Using the CRISPR/Cas9 system to epitope tag the endogenous loci of candidate proteins, we have identified Medle 2 as the first example of a host targeted protein in Cryptosporidium parvum. Medle 2 is highly polymorphic and localizes to the cytoplasm of infected HCT-8 cells in tissue culture, and to the cytoplasm of intestinal epithelial cells in infected mice. The protein is not apparent in sporozoites, but is detectable in host cells as early as 6 hours post infection. This suggests a rhoptry-independent delivery system that is assembled by the trophozoite only after invasion.

Pharmacological and imaging experiments support this view. Our current work aims to uncover the function of Medle 2 during C. parvum infection through cell biological, transcriptional and functional proteomic studies.

Session IV: Sex and Mosquitoes

Chairs: Jaime Adam-Gallego and Catherine Lavazec

Monday, September 10, 2018
1:00 pm – 2:45 pm

FT1: Mosquito reproductive factors affecting *Anopheles-Plasmodium* interactions

Perrine Marcenac *(Harvard T. H. Chan School of Public Health), Kristine L. Werling (Harvard T. H. Chan School of Public Health), Duo Peng (Harvard T. H. Chan School of Public Health), Adam South (Harvard T. H. Chan School of Public Health), Evdoxia G. Kakani (Harvard T. H. Chan School of Public Health), Sara N. Mitchell (Harvard T. H. Chan School of Public Health), W. Robert Shaw (Harvard T. H. Chan School of Public Health), Kathleen A. Westervelt (Harvard T. H. Chan School of Public Health), Serge R. Yerbanga (Institut de Recherche en Sciences de la Santé/Centre Muraz), Thierry Lefèvre (Institut de Recherche pour le Développement), Abdoulaye Diabaté (Institut de Recherche en Sciences de la Santé/Centre Muraz), Flaminia Catteruccia (Harvard T. H. Chan School of Public Health)*

The reproductive fitness of *Anopheles* mosquitoes is an important component of vectorial capacity, as it ensures large densities for the transmission of *Plasmodium* parasites. In *Anopheles gambiae,* female reproduction is profoundly affected by male-female molecular interactions regulated by the steroid hormone 20-hydroxyecdysone (20E). Upon sexual transfer, male 20E induces vast signaling cascades that permanently reshape female physiology, impacting blood feeding-induced processes that affect egg development. One of the factors regulated by male 20E transfer is the Mating-Induced Stimulator of Oogenesis (MISO), a female protein that boosts fecundity possibly via the regulation of vitellogenic lipid transporters. Here we show how the human malaria parasite *Plasmodium falciparum* exploits the physiological environment created by mating and blood feeding in the female *Anopheles* to optimize its own transmission while minimizing fitness costs to its vector. Mosquitoes that produce high number of eggs also support higher infection loads, unveiling a positive correlation between fecundity and *P. falciparum* infection intensity. However, silencing *MISO* disrupts this positive correlation and induces a fitness cost to infection, leading to decreased egg production in infected females. Correlation network analysis suggests MISO may be involved in conserved molting signaling pathways, providing new insight into molecular processes important for egg development in *A.*

gambiae. Interestingly, male 20E transfer has evolved specifically in the anopheline lineage and is highly prevalent in African malaria vector species, suggesting that the evolution of a mating system based on transfer of this steroid hormone may have contributed to *Anopheles* tolerance to *Plasmodium* infections. These results identify new molecular pathways critical to *A. gambiae* vectorial capacity that may have been shaped by evolutionary pressures from *Plasmodium*.

FT2: A mosquito salivary gland protein partially inhibits Plasmodium sporozoite cell traversal and transmission

Yang, Jing (Yale University School of Medicine)*; Schleicher, Tyler (Yale University School of Medicine); Freudzon, Marianna (Yale University School of Medicine); Rembisz, Alison (Yale University School of Medicine); Craft, Samuel (Yale University School of Medicine); Hamilton, Madeleine (Yale University School of Medicine); Graham, Morven (Yale University School of Medicine); Mlambo, Godfree (Johns Hopkins University); Tripathi, Abhai (Johns Hopkins University); Li, Yue (Yale University School of Medicine); Cresswell, Peter (Yale University School of Medicine); Sinnis, Photini (Johns Hopkins University); Dimopoulos, George (Johns Hopkins University); Fikrig, Erol (Yale University School of Medicine)*

The key step during the initiation of malaria is for motile Plasmodium parasites to exit the host skin and infect the liver. During transmission, the parasites in the form of sporozoites, are injected together with mosquito saliva into the skin. However, the contribution of vector saliva to sporozoite activity during the establishment of the initial infection of the liver is poorly understood. Here we identify a vector protein by mass spectrometry, with similarity to the human gamma interferon inducible thiol reductase (GILT), that is associated with saliva sporozoites of infected Anopheles mosquitoes and has a negative impact on the speed and cell traversal activity of Plasmodium. This protein, referred to as mosquito GILT (mosGILT) represents an example of a protein found in mosquito saliva that may negatively influence sporozoite movement in the host and may lead to new approaches to prevent malaria.

FT3: A systematic knockout screen in Plasmodium berghei identifies sex-specific regulators of gametocyte differentiation

Bushell, Ellen (Wellcome Sanger Institute); *Sanderson, Theo (Wellcome Sanger Institute); Talman, Arthur (Wellcome Sanger Institute); Russell, Andrew (Wellcome Sanger Centre); Montandon, Ruddy (Wellcome Sanger Institute); Modrzynska, Kasia (Glasgow University); Metcalf, Tom (Wellcome Sanger Institute); Bronner-Anar, Burcu (Wellcome Sanger Institute); Herd, Colin (Wellcome Sanger Institute); Kent, Robyn (Glasgow University); Girling, Gareth (Wellcome Sanger Institute); Schwach, Frank (Wellcome Sanger Institute); Rayner, Julian C (Wellcome Sanger Institute); Waters, Andrew P (Wellcome Sanger Institute); Lawnizcak, Mara K (Wellcome Sanger Institute); Billker, Oliver (Wellcome Sanger Institute)*

Plasmodium gametocyte formation relies on the master regulator AP2-G. However, how a single transcription factor initiates the distinct developmental programmes of male and female gametocytes is unknown. Having recently developed tools to interrogate gene functions in P. berghei at genome scale, we now present a systematic screen of >1000 barcoded, viable mutants for genes required for gametocytogenesis. Using fluorescent proteins expressed from male and female specific promoters as proxies, we identify 9 mutants deficient in the female marker, 7 in the male marker and 15 in both. Most genes with sex-specific functions contain likely nucleic acid binding domains, such as zinc fingers and RNA helicase domains. These genes are further characterised by belonging to the first wave of upregulated genes in ring stage parasites when these are reprogrammed into gametocytes by induced overexpression of AP2-G, making them strong candidate regulators of differentiation. Through conventional phenotyping of cloned mutants, we confirm the screen results. Using single cell transcriptomics to phenotype developmental mutants more deeply, we delineate the sequence of events during male and female differentiation, which are ultimately responsible for successful transmission of malaria parasite to their vectors.

FT4: Homeo Domain-like Protein 1 regulates chromatin structure and gene expression during the early stages of P. falciparum sexual development

Campelo, Riward (Weill Cornell Medicine); *Tong, Xinran (Weill Cornell Medicine); Xie, Wei (Memorial Sloan-Kettering Cancer Center); Batugedara, Gayani (University of California Riverside); Cook, Kate (University of Washington); Orchard, Lindsey (Pennsylvania State University); Llinas, Manuel (Pennsylvania State University); Noble, William (University of Washington); Le Roch, Karine (University of California, Riverside); Patel, Dinshaw (Memorial Sloan-Kettering Cancer Center); Kafsack, Björn (Weill Cornell Medicine)*

Malaria parasites undergo striking morphological changes as they transition from replicating asexual blood stages to the non-replicating gametocytes necessary for infection of the mosquito vector. This differentiation step requires the silencing and activation of a large number of stage-specific genes in a stable and coordinated fashion that is in part mediated by changes in chromatin structure. We recently identified Homeo Domain-like Protein 1, a previously uncharacterized protein containing a DNA-binding homeo-domain-like region with conservation across the Apicomplexa. Biochemical analysis of the HDP1 DNA-binding domain revealed that it binds DNA as a high affinity dimer.

Epitope-tagging of the endogenous locus revealed that HDP1 localizes to the nucleus of P. falciparum gametocytes. While targeted disruption of the pfhdp1 locus had no impact on parasite replication in erythrocytes, resulted in aberrant development and death during gametocytogenesis. Comparison of gene expression just prior to the onset of morphological changes in sexual development, revealed substantial upregulation of gene families regulated via heterochromatin-mediated silencing in asexual stages. In parallel, chromosome conformation capture analysis (HiC) revealed notable changes in chromatin organization in the absence of HDP1 expression. Our findings highlight the critical role that HDP1 plays in facilitating the changes in chromatin structure and gene expression underlying the transition from asexual blood stages to transmissible gametocytes.

FT5: NOT1-G is a novel member of the CAF1/CCR4/NOT complex that is essential for male gametocyte development and female gametocyte fertility in Plasmodium yoelii

Hart, Kevin (The Pennsylvania State University); *Walker, Michael (The Pennsylvania State University); Minns, Allen (The Pennsylvania State University); Lindner, Scott (The Pennsylvania State University)*

Plasmodium transmission between mosquitoes and mammals utilizes translational repression to allow the parasite to prepare the mRNAs it will need for the next developmental stage, and yet ensure that only the proper proteins are expressed at the right time. With relatively few known specific transcription factors to initiate gene transcription, Plasmodium parasites also regulate the stability and turnover of transcripts to provide more comprehensive gene regulation. We and others have demonstrated that members of the CAF1/CCR4/NOT complex have specific and important functions in both the development (CAF1) and host-to-vector transmission (CCR4-1) of the parasite. In model eukaryotes, NOT1, is utilized as a scaffold for its effector proteins, such as CAF1 and CCR4-1. Here, in stark contrast to essentially all other eukaryotes, we have bioinformatically identified that Plasmodium and the closely related Theileria genus encode two putative NOT1 proteins. We found that neither NOT1 nor its paralog NOT1-G are essential for Plasmodium yoelii asexual blood stage development, but that NOT1-G is essential for complete male gametocyte development. Moreover, pynot1-g- parasites only produce female/immature gametocytes that by genetic cross experiments are sterile and cannot be transmitted to mosquitoes. Comparative transcriptomics of pynot1-g- parasites show massive dysregulation of the female gametocyte transcriptome and implicate NOT1-G as an early regulator of RNA metabolism for gametocytogenesis. We have used bioinformatics-, immunofluorescence-, and proteomics-based approaches to identify differences in protein composition and key correlates of the critical functions of the NOT1-G complex. Together, we demonstrate that Plasmodium has evolved the NOT1-G paralog to be essential for gametocytogenesis and transmission, and show that it plays an important role in RNA metabolism in this transmission stage.

FT6: Plasmodium and Host microbiota: Friend or Foe?

Mukherjee, Debanjan (Instituto de Medicina Molecular, Faculadade de Medicina de Lisboa, Lisbon,)*; Chora, Angelo (Instituto de Medicina Molecular, Faculadade de Medicina de Lisboa, Lisbon,); Ramiro, Ricardo (Instituto de Gulbenkian de Ciancia, Oeiras, Portugal); Gordo, Isabel (Instituto de Gulbenkian de Ciancia, Oeiras, Portugal); Mota, Maria (Instituto de Medicina Molecular, Faculadade de Medicina de Lisboa, Lisbon,)*

In malaria endemic areas, Plasmodium, the causative agent of malaria, and bacterial co-infections are highly concurrent, often with fatal outcomes like cerebral malaria(CM), acute lung injury (ALI) and severe anemia. Recently, it has been shown in patients from Sub-Saharan Africa that Plasmodium infection predisposes to high bacteremia. However, the causal link between Plasmodium infection and increased bacteremia and the putative mechanisms involved behind severe malaria associated pathology is currently unknown. In the present study, using different models of rodent malaria we show that infection with Plasmodium parasites causes an increase in the bacterial diversity in the lung specifically of mice that die of ALI. Moreover, we show by genetic knockout and complementation studies that this increase in the tissue specific bacterial load was dependent on parasite sequestration in the lung.

Metagenomics analysis of the bacterial community showed marked alterations in the lung microbiome of mice that die of ALI. We report here that this increase in the bacterial burden is not associated with increased intestinal permeability or with histological alterations in the intestinal barrier. These alterations in the lung microbiome was significantly correlated with high levels of alveolar inflammation (IL-6, IL-10, IFN-γ and TNF-α). To furthermore show causality, we show that this bacterial increase in burden could be abrogated by preventing inflammation per se. Interestingly, targeting specific microbiota of the lung with antibiotics prevented malaria associated ALI in mice and prolonged survival. All these results suggest that Plasmodium blood stage-mediated immunomodulation may result in aberrant expansion of bacteria in the lungs during malaria infection and also pave the way for interventional studies for malaria associated ALI.

TT1: Breaking the proventricular bottleneck: characterization of genes that enhance trypanosome migration to the tsetse salivary glands

Casas-Sanchez, Aitor (Liverpool School of Tropical Medicine); *Lopez-Escobar, Lara; Cansado-Utrilla, Cintia; Haines, Lee; Walrad, Pegine; Acosta-Serrano, Alvaro*

During its life cycle in the tsetse vector, Trypanosoma brucei encounters many challenges involving colonization of several tsetse tissues. These are accompanied by a series of developmental changes that are ultimately the result of tightly programmed gene expression. After mammalian 'stumpy' bloodstream forms differentiate into the insect procyclic stage, the parasites first colonize the tsetse midgut ectoperitrophic space and then invade the proventriculus, where differentiation into epimastigotes occurs. The last step of this journey involves migration to and establishment of a salivary gland infection, which ensures transmission of infectious metacyclic trypomastigotes during tsetse blood feeding. To understand which T. brucei genes are crucial for the successful life cycle completion in the tsetse, we used RNA-seq to compare the gene expression profiles of a fly-transmissible T. brucei strain with that of a strain unable to infect tsetse salivary glands. We found >700 transcripts up regulated in the fly-transmissible strain. Among the top hits we identified and characterized were several folate transporters, a glutamate dehydrogenase and the RNA-binding protein RBP6 known for triggering differentiation of procyclics to metacyclics when overexpressed in vitro. The top hit was a hypothetical protein with a predicted zinc finger domain, which we found to be 7-fold up regulated in proventricular trypanosomes (transmissible strain only) at late stages of infection. Furthermore, the overexpression of this hypothetical protein in the transmission-impaired strain restored life cycle completion in the tsetse.

TT2: Nutritional Supplementation Enhances Innate Immune Response Against Plasmodium Liver Stage Parasites

Meireles, Patricia (Instituto de Medicina Molecular Joao Lobo Antunes); *Bras, Daniela; Mendes, Antonio; Fontinha, Diana; Andrade, Carolina; Prudencio, Miguel*

Plasmodium parasites, the causative agents of malaria, scavenge nutrients from the host in order to sustain their replication. Thus, it is not surprising that host deficiencies in several nutrients, such as glucose, lipids, iron and amino acids can highly impact infection and disease. Recently, we have unraveled a vital role for arginine (Arg, R) metabolism during Plasmodium liver infection. Arg is the main synthetic precursor of polyamines and nitric oxide (NO), whose role in numerous infections has been widely studied. Arg is becoming increasingly popular in nutritional supplementation as an enhancer of immune response, being linked to proliferation of T lymphocytes, activation of macrophages and increased antibody production. Arg supplementation in the context of malaria enhances NO production, decreases parasitaemia and improves survival in laboratory models, but results from the clinic remain unclear. Our data shows that RKV supplementation, which combines Arg with two amino acids that interfere with Arg metabolism, lysine (K) and valine (V), strikingly decreases the liver load of Plasmodium infection. Although intra-hepatic parasites display impaired growth, their vast majority is eliminated in the context of RKV supplementation, suggesting a boost of the host's immune response. Employing knockout mice and in vivo administration of depleting antibodies we have restricted the key innate immune cells involved in RKV-dependent parasite elimination to Natural Killer (NK) cells, commonly used in immunotherapies against cancer and viral infections, and Innate Lymphoid Cells (ILCs), whose role remains unclear. Employing RKV supplementation we are unveiling a new and very efficacious immune mechanism against Plasmodium liver infection that has the potential to be stimulated, and therefore constitutes a novel target for prophylactic therapies for malaria.

Session V: I like to Move It

Chairs: Aoife Heaslip and Hangjun Ke

Tuesday, September 11, 2018

9:00 am – 11:35 am

FT1: Repeat region of the circumsporozoite protein has a functional role in Plasmodium sporozoite motility

Balaban, Amanda (Johns Hopkins Bloomberg School of Public Health)*; Mitra, Jaba (University of Illinois Urbana-Champaign); Gregory, Jason (Johns Hopkins Bloomberg School of Public Health); Vartak, Natasha (Johns Hopkins Bloomberg School of Public Health); Sinnis-Bourozikas, Ariadne (Johns Hopkins Bloomberg School of Public Health); Shears, Melanie (Johns Hopkins Bloomberg School of Public Health); Ha, Taekjip (Johns Hopkins University); Frischknecht, Friedrich (Heidelberg University Medical School); Sinnis, Photini (Johns Hopkins Bloomberg School of Public Health)*

Plasmodium sporozoites are the infective stage of the malaria parasite and utilize a substrate based gliding motility to establish infection in the mammalian host. The major surface protein of sporozoites, the circumsporozoite protein (CSP) is composed of a central tandem-repeat flanked by an N-terminal domain and a C-terminal adhesion domain. Previous studies have elucidated the roles of the flanking domains but little is known about repeat region, despite its being the target of protective antibodies. Using the rodent malaria parasite, Plasmodium berghei, we generated a panel of CSP repeat mutants to determine its functional role in malaria infection. Our results demonstrate that the repeat region is essential for parasite migration in both the mosquito and mammalian hosts. Further analysis demonstrated that CSP repeat mutants in which the repeats were scrambled or significantly truncated, were unable to engage in productive circular gliding motility and instead exhibited abnormal patch gliding behavior. Using reflection interference contrast microscopy (RICM), we found that the defects in parasite motility were likely due to perturbed adhesion site assembly and turnover, a finding supported by our observation that TRAP containing adhesion sites are altered on these mutants. By using single molecule force spectroscopy experiments, we also found that the CSP repeats have elastic properties which can be perturbed by altering the amino acid sequence, suggesting that the repeats play a mechanical role for sporozoites during migration. Our data shows that the repeat region has functional properties previously unappreciated and provides new insights into the mechanisms of sporozoite motility.

FT2: An alveolate conserved mechanism is implicated in rhoptries secretion event during Apicomplexa invasion

***Aquilini, Eleonora (Montpellier University /CNRS)**; Dos Santos Pacheco, Nicolas (Universite de Montpellier); Suarez, Catherine (Universite de Montpellier); Maynadier, Marjorie (Universite de Montpellier); Lebrun, Maryse (Universite de Montpellier)*

Host-cell invasion by apicomplexan parasites is governed by coordinated and sequential exocytosis of specialized apical organelles: micronemes and rhoptries. Despite decades of research on parasite invasion our knowledge on membrane fusion events and exocytosis mechanisms of these organelles remains largely unknown. Together with ciliates and dinoflagellates, apicomplexan parasites belong to the Alveolata superphylum. Although morphologically different, alveolates share several features, including the presence of secretory organelles. Previous studies in Paramecium tetraurelia showed that their defensive extrusive organelles, termed trichocysts, have a characteristic arrangement of particles on the membrane over the docking sites: a ring of intramembranous particles with a “rosette” of 8-10 particles in the center. Rosette assembly is required for membranes fusion and, was shown to be essential for trichocyst secretion upon stimulation. Remarkably, a similar structure was observed at the apex of several apicomplexan parasites, which has remained uncharacterized to date. The dissection of mutants lacking the rosette and defective for trichocyst exocytosis (called ND for “non-discharge”) prompted the identification of essential components of the trichocyst-membrane fusion machinery in Paramecium. We have identified, localized and characterized the orthologs of nd6 and nd9 in the apicomplexan model, Toxoplasma gondii. While TgND6 localizes to the apical pole of the parasites, TgND9 is located in the cytoplasm. Conditional depletion of TgND6 and TgND9 didn’t affect parasites intracellular replication, egress or motility. Importantly, micronemes secretion also remains unaffected, but depletion of TgND6 or TgND9 abolishes rhoptry secretion and blocks the host-cell invasion process. This study identifies novel proteins essential for rhoptries secretion and, supports the hypothesis of an alveolate conserved mechanism for organelles-dependent membrane fusion events.

FT3: Combining Reverse Genetics and Three-dimensional Electron Microscopy to Dissect Plasmodium knowlesi Invasion of Red Blood Cells

Hart, Melissa (LSHTM)*; Mohring, Franziska (LSHTM); Charleston, James (LSHTM); Thomas, James (LSHTM); Saibil, Helen (Birkbeck, University of London); Moon, Robert (LSHTM)*

The symptoms of malaria are caused by Plasmodium invasion of red blood cells, which represents a potentially key drug and vaccine targetable stage of the parasite's life cycle. However, the precise cellular and molecular mechanisms behind this complex biological process are yet to be fully understood. Two major protein families, the erythrocyte binding proteins (EBPs) and the reticulocyte binding like proteins (RBLs) have been studied extensively in P. falciparum and are hypothesized to have overlapping, but critical roles either just prior to or during the formation of the tight junction (TJ) between parasite and host cell during invasion. The zoonotic malaria parasite, P. knowlesi, recently adapted to culture in human cells, is much more genetically amenable than P. falciparum, and contains a smaller repertoire of EBP and RBL members, which have less functional redundancy. In particular, one EBP (Duffy binding protein alpha, DBPα) and one RBL (Normocyte binding protein Xa, NBPXa) have previously shown to be essential for invasion of human red blood cells. By taking advantage of the unique biological features of P. knowlesi, and newly adapted techniques for CRISPR-Cas9 genome editing, we have undertaken a comprehensive genetic analysis of the EBP /RBL families and combined this with live microscopic analysis and electron tomography to unravel their role during invasion. By simultaneously tagging and knocking out multiple family members and associated invasion genes within the same parasite lines we have begun to build a picture of the precise order of events leading to invasion, such as organelle secretion timing, and localisation of family members throughout invasion.

FT4: It Takes Two to Tango: the P52/P36 Hepatocyte Invasion Complex

Arredondo, Silvia A. (Center for Infectious Disease Research); *Swearingen, Kristian E. (Institute for Systems Biology); Martinson, Thomas (Center for Infectious Disease Research); Steel, Ryan (Center for Infectious Disease Research); Dankwa, Dorender A. (Center for Infectious Disease Research); Harupa, Anke (Center for Infectious Disease Research); Camargo, Nelly (Center for Infectious Disease Research); Betz, William (Center for Infectious Disease Research); Vigdorovich, Vladimir (Center for Infectious Disease Research); Oliver, Brian G. (Center for Infectious Disease Research); Ishino, Tomoko (Ehime University); Kangwanrangsan, Niwat (Ehime University); Sather, Noah (Center for Infectious Disease Research); Mikolajczak, Sebastian (Center for Infectious Disease Research); Vaughan, Ashley M. (Center for Infectious Disease Research); Torii, Motomi (Ehime University); Moritz, Robert L. (Institute for Systems Biology); Kappe, Stefan H. I. (Center for Infectious Disease Research)*

Following an infected mosquito bite, a limited number of Plasmodium sporozoites will make their way to the liver to continue the cycle of infection in the mammalian host. Once in the liver, sporozoites traverse cells searching for a “suitable” hepatocyte, invading these cells through a process that results in the formation of a parasitophorous vacuole (PV), a protective compartment in which the parasite undergoes intracellular replication as a liver stage. Previous studies have established that two members of the Plasmodium s48/45 protein family, P36 and P52, are essential for productive invasion of host hepatocytes as their simultaneous deletion results in growth-arrested parasites lacking a PV. Recent studies point towards a pathway of entry possibly involving the interaction of P36 with hepatocyte receptors EphA2, CD81 and SR-B1. However, the relationship between P36 and P52 during invasion remains unknown. Here we show that parasites with a single P52 or P36 gene deletion lack a PV after hepatocyte invasion, thereby each pheno-copying the P52/P36 dual gene deletion parasite line and indicating that both proteins are important in the establishment of a PV and act in the same pathway. We then created a Plasmodium yoelii P36mCherry tagged parasite line that allowed us to visualize the subcellular localization of P36 in the sporozoite secretory microneme

organelles and found that it co-localizes with P52. Furthermore, through co-immunoprecipitation studies in vivo, we determined that P36 and P52 form a protein complex in sporozoites, implying a concerted function for both proteins within the PV formation pathway. In addition, we found that P36 is secreted from the sporozoite. Our results support a model in which glycophosphatidylinositol (GPI)-anchored P52 may serve as a scaffold to facilitate the interaction of secreted P36 with host receptors.

TT1: DOC2 domain proteins in Toxoplasma gondii Ca2+-dependent secretion

Tagoe, Daniel A. (Boston College); *Coleman, Bradley; Stoneburner, Emily; Drozda, Allison; Coppens, Isabelle; Gubbels, Marc-Jan*

The central and essential role of Ca2+ in triggering the release of secretory proteins from the apical microneme organelles of the obligate intracellular parasite Toxoplasma gondii during the lytic cycle is unequivocal. Proteins with Double C2 (DOC2) domains are known Ca2+ sensors mediating protein secretion across eukaryotes. Of the five known DOC2 protein families, only two are represented in the Toxoplasma genome: 3 ferlin family proteins (FER1-3) and one unconventional protein we named TgDOC2. To decipher the functions and mechanism of these DOC2 protein encoding genes we are generating mutants. A temperature sensitive mutation in TgDOC2 abrogates microneme secretion. We used quantitative mass spectrometry (SILAC) to define the secreted microneme proteome in tachyzoites. We confirmed the microneme secretion defect is specific and identified one new protein released in a microneme dependent fashion and one protein with enhanced release kinetics upon blocked microneme secretion. Progress in assessment of these proteins will be presented. Overexpression of a dominant negative FER1 allele led to mislocalization of fully processed microneme proteins in an apical compartment. On the other hand, overexpressing of full length FER1 led to a phenotype consistent with constitutive microneme secretion. Both these phenotypes cause a growth defect. Together, these data suggest of role for FER1 in microneme, targeting, tethering and/or secretion. We recently showed that FER2 functions in rhoptry secretion. Interestingly, a complete gene knock-out of FER3 resulted in increased plaque number and size; detailed phenotypic characterization is in progress. Finally, we are applying proximity based biotinylation (BioID2) to map the molecular context in which the Ferlins and TgDOC2 facilitate organellar secretion in the lytic cycle.

TT2: Blood donor variability as a modulatory factor in Plasmodium falciparum invasion phenotyping assays

Thiam, Laty G. (West African Centre for Cell Biology of Infectious Pathogens); *Aniweh, Yaw; kusi, Kwadwo A.; Niang, Makhtar; Gwira, Theresa M.; Awandare, Gordon A.*

Plasmodium falciparum uses multiple ligand-receptor interactions to invade erythrocytes. Blood stage malaria vaccines mainly target antigens involved in erythrocytes invasion. Thus, unraveling the nature of ligand-receptor interactions involved in invasion is crucial in malaria vaccine development.
Conducting large-scale P. falciparum phenotyping studies inevitably involves the use of blood from different donors, which could affect the outcome of invasion inhibitory assays. However, the effect of blood donor variability in characterizing P. falciparum phenotypic diversity remains unaddressed.
Therefore, we are currently investigating variations in invasion efficiency/phenotype observed using different donor erythrocytes. Erythrocytes were treated with different enzymes, labeled with a fluorescent dye and used in P. falciparum standard invasion assays. The percentage of invasion was determined by flow cytometry and for all assays, the parasite's invasion phenotype was adjudged by comparing invasion in untreated and enzyme-treated acceptor cells. Our data show that invasion efficiency of both P. falciparum clinical isolates and laboratory adapted strains is affected by the nature of the acceptor erythrocytes. However, the clinical isolates tested here, in spite of all using the sialic acid independent pathway, show more diverse invasion patterns. Interestingly, our preliminary data suggest that hemoglobin genotypes influence parasite invasion efficiencies. Additionally, our data show that the sensitivities to enzyme treatment seemed to be driven by the receptor density on the donor erythrocyte surface. This suggests that, like the parasite's genetic make-up, the intrinsic properties of target erythrocytes may also play a role in the observed invasion phenotype. In conclusion, the data show that blood donor variability is a modulatory factor influencing P. falciparum invasion efficiency and should be an important consideration in invasion phenotyping assays.

FT5: Lysosome exocytosis by hepatocytes is pivotal to Plasmodium infection

Vijayan, Kamalakannan (Center for Infectious Disease Research); *Cestari, Igor (Center for Infectious Disease Research); Mast, Fred (Center for Infectious Disease Research); Glennon, Elizabeth (Center for Infectious Disease Research); Kain, Heather (Center for Infectious Disease Research); Brokaw, Alyssa (Department of Global Health, University of Washington); Aitchinson, John (Center for Infectious Disease Research); Stuart, Kenneth (Center for Infectious Disease Research); Kaushansky, Alexis (Center for Infectious Disease Research)*

The invasion of a suitable host hepatocyte by mosquito-transmitted Plasmodium sporozoites is an essential early step in successful malaria parasite infection. While several host and parasite factors have been identified to contribute to the entry process, the biochemical and cellular events that facilitate sporozoite entry remain largely unknown. Here, we demonstrate that lysosome exocytosis is critical for sporozoite entry of hepatocytes. Infected hepatocytes exhibit a higher portion of their lysosomes away from the nucleus, and elevated levels of LAMP1 on the surface of the cell. Lysosome exocytosis is not dependent on cell traversal activity of the sporozoite, as SPECT2- parasites, which are traversal deficient, exhibit surface-exposed LAMP1 and scattered lysosomes. Sporozoites actively sequester and fuse with the host lysosome as evident from the co-localization of punctate to a smooth pattern of lysosomes on the surface of the internalized sporozoites. Knockdown of Syt7, a protein involved in lysosome fusion to the plasma membrane, dramatically reduces Plasmodium infection. In contrast, inducing fusion of lysosomes with the plasma membrane using the small molecule ionomycin dramatically increases sporozoite infection. Interestingly, genetic or pharmacological perturbations that alter lysosome exocytosis impact infection rates by Plasmodium sporozoites and Typanosoma cruzi parasites, but small molecules that alter lysosome trafficking and exocytosis have little to no impact on Toxoplasma gondii infection. These data reveal novel insights into the mechanism of cell entry for Plasmodium sporozoites, and also call into question how evolutionary relatedness contributes to the mechanisms of pathogen entry.

FT6: The Great Escape: investigating the role of SERA6 in malaria parasite egress from the red blood cell

Tan, Michele Ser Ying (Francis Crick Institute); *Thomas, James A. (London School of Hygiene & Tropical Medicine); Borg, Aaron (Francis Crick Institute); Howell, Steven (Francis Crick Institute); Snijders, Ambrosius P. (Francis Crick Institute); Blackman, Michael J. (Francis Crick Institute & London School of Hygiene & Tropical Medicine)*

Malaria is a devastating parasitic disease. Resistance of the most dangerous causative agent, Plasmodium falciparum, to frontline antimalarial drugs necessitates an improved understanding of parasite biology to facilitate new approaches to disease control or eradication. Malaria pathogenesis arises from replication of asexual blood stages in the human host. Merozoites invade erythrocytes, replicate within a parasitophorous vacuole (PV) then subsequently burst out of and destroy the erythrocyte in a process called egress. The released merozoites immediately invade fresh erythrocytes to repeat the cycle. Malaria parasite egress is a key biological pathway with much potential for identification of novel drug targets. Work over several years has shown that egress is tightly controlled by a parasite enzyme cascade in which activation of the single cGMP-dependent protein kinase PKG triggers the discharge of a parasite serine protease called SUB1 into the lumen of the PV. SUB1 in turn proteolytically processes a PV-located cysteine protease called SERA6 in P. falciparum. Whilst SUB1 is required for all the morphological changes that lead up to egress, including PV membrane rupture, SERA6 is essential only for erythrocyte membrane rupture, the final step in egress. However, the exact molecular mechanisms underlying the role of SERA6 in egress remain a mystery. Here we present recent insights into the regulation of SERA6 activity and how the enzymatic function of SERA6 leads to cleavage of erythrocyte cytoskeletal components, resulting in its collapse and eventual lysis of the infected erythrocyte.

FT7: Malaria Parasite Egress Program: Vacuolar Rounding, Exonemal Activation of Membrane Rupture and Degradation

Glushakova, Svetlana (SIB/NIH/NICHD, Bethesda, MD); *Beck, Josh R (Washington University School of Medicine, St. Louis, MO; Department of Biomedical Sciences, Iowa State University, Ames, IA); Garten, Matthias (SIB/NICHD/NIH, Bethesda, MD); Busse, Brad L. (SIB/NICHD/NIH, Bethesda, MD); Nasamu, Armiyaw S. (Washington University School of Medicine, St. Louis, MO); Tenkova-Heuser, Tatyana (SIB/NICHD/NIH, Bethesda, MD); Heuser, John (SIB/NICHD/NIH, Bethesda, MD); Goldberg, Daniel (Washington University School of Medicine, St. Louis, MO); Zimmerberg, Joshua (SIB/NICHD/NIH, Bethesda, MD)*

During intraerythrocytic replication, Plasmodium falciparum is isolated from its host red blood cytoplasm by the parasitophorous vacuole membrane (PVM). Both the PVM and the parasite plasma membrane (PPM) must be breached by the time of egress to release the parasite to the blood stream and disseminate infection. The molecular mechanism of egress relies on a set of kinases and proteases, regulated by intracellular messengers such as parasite [Ca2+free]. Spatial and temporal coordination of this mechanism with relation to the degradation of the two limiting membranes is critical to define the targets of the different molecules of the egress program.Using time-lapse super-resolution confocal microscopy, we imaged individual red blood cells infected with Plasmodium falciparum (and enriched for late schizonts) having differential fluorescence labeling of the PVM and PV lumen; the red blood cell plasma membrane was detected by scanning differential interference contrast. Application of inhibitors then allowed us to link clearly sequential, morphologically definable stages of the egress pathway. A new obligatory stage in egress was discovered, as the PVM rounded minutes before egress under control of parasite intracellular calcium. After ~1.5 minutes, under control of PfPKG and SUB1, the parasitophorous vacuole ruptured in a discrete region and subsequently deteriorated progressively over the next ~6 minutes. Rupture lead to release of vacuolar contents and initiated red blood cell membrane distortion, lasting until the last minute before egress. The newly-formed parasites then mobilize and the red cell permeabilize just before the cascade of events culminates in egress.Taken together, dissection of the membrane degradation events gives us a framework to classify involved and yet undiscovered processes leading to egress.

TT3: A novel role for EXP2 in invasion of Plasmodium sporozoites

Mello-Vieira, Joao (Instituto de Medicina Molecular João Lobo Antunes); *de Koning-Ward, Tania; Mota, Maria; Zuzarte-Luís, Vanessa*

Plasmodium spp. are intracellular parasites that infect vertebrates, causing the disease called malaria. Parasites first infect hepatocytes and later infect and replicate repeatedly inside erythrocytes. In both stages of the infection, parasites develop inside a parasitophorous vacuole (PV) limited by a host-derived membrane (PVM). During the blood stage (BS), Plasmodium parasites translocate their proteins outside of this vacuole via a multi-protein complex. Exported Protein 2 (EXP2) is an essential component of this complex. EXP2 was also reported to be present at the PVM during the liver stage (LS) of infection, although its function is still unknown. To assess EXP2 function in the LS, we used a conditional knock-out (cKO) parasite line. Surprisingly, EXP2 cKO sporozoites (the liver infectious form of the parasite) are impaired in invading the hepatocyte, yet develop normally once inside it. In fact, wild-type sporozoites express EXP2 in the cytosol, however, upon transition to the mammalian environment (exposure to serum and increase in temperature), EXP2 is translocated to the membrane of the sporozoite. Importantly, supplementing EXP2 cKO parasites with recombinant EXP2 protein during the invasion process, rescues their invasion defect. We hypothesize that the secreted EXP2 protein, creates pores at the membrane of the hepatocyte, which induce endocytosis by the host cell, allowing for the successful invasion of the parasite. Indeed, stimulating endocytosis, also rescues the defect of the EXP2 cKO parasites. Altogether, we propose a new role for a well-characterized BS protein, that is critical for invasion of Plasmodium sporozoites. This mechanism highlights the importance of the host cell and host cellular processes that are manipulated by the parasite.

TT4: Impact of malaria-protective glycophorin polymorphism on Plasmodium falciparum invasion

***Kariuki, Silvia N. (KEMRI-Wellcome Trust Research Programme); Kariuki, Silvia N.**; Marin-Menendez, Alejandro; Leffler, Ellen; Band, Gavin; Rockett, Kirk; Macharia, Alex; Makale, Johnstone; Nyamu, Wilfred; Ndung'u, Francis; Kwiatkowski, Dominic; Williams, Thomas; Rayner, Julian*

Mortality rates from malaria remain high especially in resource-poor areas of sub-Saharan Africa. The repeated emergence and spread of drug-resistant Plasmodium falciparum parasites means that finding new approaches for combating the disease is of growing importance. A number of human genetic polymorphisms have recently been identified that are associated with protection against severe malaria, most of which are found in or near genes encoding proteins important for the structure and function of red blood cells (RBCs), the primary host cell for P. falciparum parasites. These include a novel complex structural variant in the glycophorin gene cluster that encodes the Dantu blood group antigen which confers high-level protection against severe malaria. The mechanisms of protection conferred by this and other polymorphisms remain unknown. We investigated the impact of the Dantu polymorphism on P. falciparum RBC invasion and RBC membrane protein expression through flow cytometry-based in vitro assays. We collected RBC samples across genotype groups from a cohort of Kenyan children for the in vitro assays. We observed a strong and linear reduction in the ability of multiple P. falciparum strains to invade RBCs from Dantu heterozygote and homozygotes respectively. We also observed a significant reduction in expression of glycophorin A (GYPA) and glycophorin B (GYPB), and a significant increase in glycophorin C (GYPC) expression on the surface of RBCs from Dantu heterozygotes and homozygotes. The reduction in invasion observed in carriers of the Dantu variant allele indicates that this polymorphism could confer protection against malaria infection by significantly reducing parasite invasion into the RBC, perhaps mediated by altered expression of the glycophorin receptors on the RBC membrane. Ongoing studies include video microscopy to monitor the invasion process, and testing genetically manipulated P. falciparum strains where specific invasion ligands have been deleted.

TT5: Comparative assessment of PbSLTRiP (Sporozoite and Liver stage expressed Tryptophan Rich Protein) peptides as vaccine candidates against Plasmodium berghei in mice.

Quadiri, Afshana (National Institute of Immunology); *Singh, Agam Prasad*

An effective vaccine is required to inhibit malaria prevalence and its transmission. Identifying the key immunogenic antigens that can be potential vaccine candidates and understanding the interactions of these proteins with host proteins to modulate host forms the key focus of our laboratory. Earlier our laboratory showed that SLTRiP) conferred protection to liver stage of Plasmodium growth in rodents. Here we demonstrate the protection is mainly due to cell mediated immune responses and PbSLTRiP specific cellular memory responses could be recalled in mice challenged with P. berghei parasites even after a year post immunisation. Following the immune and memory responses, we proceeded to define specific T-cell stimulatory peptides for their inclusion in future vaccines. For this we designed a panel of 9-15 mer synthetic peptides representing nearly the whole protective fragment of protein and identified MHC class I epitopes that are highly immunogenic in nature. The peptide selection was done by expressing and testing multiple fragments of PbSLTRiP protein and selecting peptides from protective fragments by using T-epitope based prediction algorithm. The responses generated by these peptides were compared to identify the immunodominant peptides. We utilized both the IFNγ enzyme-linked immunosorbent spot assay and intracellular cytokine staining to achieve this. We identified immunodominant peptide responses for CD8+ T cells in C57BL/6 mice and these immunodominant peptides were used to immunize mice. We found that the better immunological profile of these epitopes translated into a higher efficacy against malaria challenge in mice. We also characterized the polyfunctionality of induced immune responses. These findings contribute to our understanding of the immunological mechanisms underlying the protective vaccines. Our findings provide a basis for assessment of these peptides for their inclusion in clinical vaccine constructs against malaria.

Session VI: Bad to the Bone

Chairs: Prakash Srinivasan and Taco Kooij

Tuesday, September 11, 2018

1:00 pm – 1:50 pm

FT1: Stuck on You: The Plasmodium basal complex is required for proper daughter cell segmentation

Rudlaff, Rachel M. (Harvard Medical School)*; Streva, Vincent A. (Division of Infectious Diseases, Boston Children's Hospital); Dvorin, Jeffrey D. (Division of Infectious Diseases, Boston Children's Hospital)*

During the blood stage of malaria infection, Plasmodium parasites must asexually divide via schizogony during each 48-hour life cycle. Over the course of schizogony, a single parasite produces 20 to 30 daughter cells within a common cytoplasm that form into invasive merozoites by segmentation. The basal complex is hypothesized to be important for segmentation by 1) forming a contractile ring around nascent daughter cells, 2) connecting the inner membrane complex to the parasite plasma membrane, and 3) mediating membrane fusion to “clip” daughter cells from the residual body, completing cytokinesis. We identified a Plasmodium protein with no previously known function that is a novel basal complex protein, PF3D7_0407800, which we named coordinator of nascen-t cell detachment, or PfCINCH. Using CRISPR-Cas9 we generated a parasite line where PfCINCH can be inducibly knocked down with the TetR-DOZI system and determined that PfCINCH is required for asexual replication. By time-lapse microscopy, super-resolution fluorescence microscopy, and transmission electron microscopy we observed that parasites deficient in PfCINCH mature normally throughout the majority of the asexual life cycle and even egress at 48 hours post invasion; however, daughter cells contain multiple nuclei and sets of organelles in a single parasite plasma membrane. Additionally, by focused ion beam – scanning electron microscopy (FIB-SEM) we elucidated the 3D structure of these mutants and observed that many of these agglomerated cells remain unseparated from each other at their basal ends. Taken together, this suggests that the Plasmodium basal complex is required for organizing budding daughter cells and pinching these cells off from the residual body. Finally, by immunoprecipitating PfCINCH complexes we have identified several novel members of the Plasmodium basal complex. Therefore, in this work we have applied several state of the art techniques to describe the function of a previously uncharacterized protein and shed light on the protein components of Plasmodium segmentation machinery. ------

FT2: The dually localised actin regulator TgCAP is important for controlling a subset of actin-dependent processes, including cell-cell communication, motility and organisation within the host-cell

Hunt, Alex (Francis Crick Institute)*; Kent, Robyn (University of Vermont); Carmeille, Romain (University of Connecticut); Russell, Matt (Francis Crick Institute); Wagener, Jeanette (Francis Crick Institute); Heaslip, Aoife (University of Connecticut); Ward, Gary (University of Vermont); Treeck, Moritz (Francis Crick Institute)*

Toxoplasma gondii progression though the lytic cycle depends on a divergent actin-myosin motor. This motor powers gliding motility which is essential for active invasion and egress. As such, regulators of actin polymerisation have been shown to play key roles in these processes. Cyclase-associated protein (CAP) is an actin-binding protein conserved across eukaryotes. In this study we investigate the role of CAP in Toxoplasma biology using a new generation of DiCre RHΔKu80 parasites where loss of DiCre activity is prevented. Through TgCAP endogenous tagging and specific antibodies we identified that TgCAP has a unique extension that is not present in most other apicomplexa, giving rise to two isoforms with distinct subcellular localisations. Conditional knockout of TgCAP led to significant defects in motility initiation, invasion and active egress, as well as impacting dense granule trafficking. Ablation of TgCAP also resulted in loss of the parasite's characteristic rosette organisation and a significant defect in intravacuolar cell-cell communication, with some parasites appearing to lose connectivity while maintaining synchronous division. Despite displaying phenotypes closely resembling the TgActin knockout, TgCAP is dispensable for in vitro culture. Strikingly, TgCAP knockout parasites do not display a loss of in vivo virulence when performed in the type-I RH strain but show a complete attenuation in the type-II Pru strain. Through complementation studies we are currently investigating the function of the membrane bound CAP isoform only present in coccidia.

FT3: Keeping in shape: The role of GAPM proteins in maintaining the structure of Toxoplasma gondii

Harding, Clare (Whitehead Institute for Biomedical Research)*; Ho Kang, Joon (Massachusetts Institute of Technology); Meissner, Markus (Ludwig-Maximilians-University of Munich); Lourido, Sebastian (Whitehead Institute of Biomeical Research)*

Microtubules are ubiquitous in eukaryotic cells and have been adapted to perform multiple functions. Single-celled protists in particular often contain elaborate microtubule structures, including arrays of microtubules at the cell periphery. In the obligate intracellular parasite Toxoplasma gondii these cortical microtubules lie beneath the alveoli and are unusually stable. Here, we show that an integral membrane protein of the alveoli, GAPM1a, has a role in maintaining microtubule stability. Conditional depletion of GAPM1a (or GAPM2a), resulted in a severe defect in cell division, reminiscent of deletion of other alveoli localized proteins. However, implementation of a degron-based depletion system allowed us to examine the effects of GAPM1a loss prior to replication. Rapid degradation of GAPM1a caused the disorganization of cortical microtubules and their depolymerization. These dramatic changes in the cortical cytoskeleton led to a rounding of the parasites, accompanied by a decrease in cellular volume and increase in density. Our results suggest that the GAPM proteins act as a link between the alveoli and cortical microtubules. This link helps to stabilize the cortical microtubules, which are required to maintain the shape of cell.

TT1: TgMyoF is an organizer of the endosome-like compartment in Toxoplasma gondii

Carmeille, Romain (University of Connecticut); *Heaslip, Aoife*

Toxoplasma gondii tachyzoites have a highly organized and polarized endomembrane system that includes three specialized secretory organelles (the micronemes, rhoptries and dense granules) and the “endosome-like compartment” (ELC) that is required for processing and sorting proteins in the endocytic and exocytic pathways. Our goal is to determine how the parasite cytoskeleton maintains the strict spatial organization of the ELC and determine how vesicles are transported to and from this compartment. Here, we identify the small GTPase Rab6 as a new component of the ELC system that occupies a sub-compartment distinct from previously identified ELC markers Rab5 and Rab7. Live cell imaging of parasites expressing EmeraldFP-Rab6 (EmFP-Rab6) demonstrates that the Rab6 compartment is highly dynamic, and Rab6 labeled vesicles are observed budding from and fusing with this compartment. In addition, Rab6 vesicles exhibited directed, motor-driven transport with a velocity of 0.92± 0.3um/s as previously observed for the dense granules. After depolymerization of filamentous actin (F-actin) with cytochalasin D, the Rab6 compartment became fragmented, static and distributed throughout the parasite body. In addition, the number of Rab6 vesicles in the cytosol decreased and those that remained no longer exhibited directed transport. A similar phenotype was observed in the absence of the myosin molecular motor TgMyoF. Rab5 and Rab7 ELC compartments also fragmented in the absence of TgMyoF and F-actin. Thus, we propose a model where TgMyoF motion along F-actin powers the formation and transport of Rab6 vesicles and this acto-myosin system preserves the apical localization and integrity of the ELC.

Session VII: Fission and Fusion

Chairs: Taco Kooij and Prakash Srinivasan

Tuesday, September 11, 2018

2:20 pm – 3:30 pm

FT1: Toward deciphering Toxoplasma. gondii endocytosis during gliding

Gras, Simon *(Ludwig-Maximilians-Universität, LMU); Jimenez-Ruiz, Elena (Ludwig-Maximilians-Universität, LMU); Lemgruber, Leandro (Wellcome Center, Glasgow); Klinger, Christen (Wellcome Center, Glasgow); Meissner, Markus (Ludwig-Maximilians-Universität, LMU)*

Toxoplasma gondii invades the host cell in an active process, involving the parasites ability move by gliding motility and invasion. While the acto-myosin-system of the parasite plays a crucial role in the formation and release of attachment sites during this process, there are still open questions, how the force powering motility is generated. A recent study in Dictyostelium demonstrates that force can be generated by an exocytic-endocytic cycle, called fountain flow model (Tanaka et al. (2017)). Similar to this model, polarised secretion of micronemes is essential for T. gondii motility. However, one main mechanism of the fountain flow model has not yet been described in extracellular tachyzoites: Endocytosis. To assay if endocytosis is involved in motility we used endocytosis inhibitors, such as Phenylarsine oxide and Trifluoroperasine and found potent inhibition of gliding motility. Similarly, retrograde flow was abrogated, suggesting the presence of an endocytic mechanism crucial for T. gondii motility. Using different lipid dyes we were able to illustrate the capacity of extracellular tachyzoites to uptake exogenous material. Moreover, we identified one of these lipids to be a potent stimulator of endocytosis using nano-gold particles (NGP). Interestingly, we observed that the NGP were trafficked via different vesicles and were found in the VAC and the rhoptries by both classical and electronic microscopy. The correlations between endocytosis, retrograde flow and gliding suggests that a motor similar to the fountain flow model is involved in T. gondii motility.Tanaka, M., T. Kikuchi, H. Uno, K. Okita, T. Kitanishi-Yumura, and S. Yumura. 2017. 'Turnover and flow of the cell membrane for cell migration', Sci Rep, 7: 12970.

FT2: Inositol Pyrophosphates Control Phosphate Release from Acidocalcisomes of Trypanosoma brucei and Vacuoles of Saccharomyces cerevisiae

Potapenko, Evgeniy (Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA)*; Cordeiro, Ciro (Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA); Huang, Guozhong (Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA); Storey, Melissa (Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA); Jessen, Henning (Department of Chemistry and Pharmacy, University of Zürich, Zürich, Switzerland); Starai, Vincent (Departments of Microbiology and Infectious Diseases, University of Georgia, Athens, GA); Docampo, Roberto (Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA)*

Acidocalcisomes of Trypanosoma brucei and the acidocalcisome-like vacuoles of yeast are acidic calcium compartments that store orthophosphate (Pi) in the form of polyphosphate (polyP). It is currently unknown how the release of Pi from these stores is regulated. Both organelles possess a phosphate sodium symporter (TbPho91, and Pho91p, respectively). TbPHO91-KO procyclic form trypanosomes had larger acidocalcisomes and increased Pi content and were more sensitive to ?-irradiation than wild type cells, suggesting a role for TbPho91 in Pi release. Functional expression of TbPHO91 or PHO91 in Xenopus laevis oocytes followed by two-electrode voltage clamp recordings showed that application of pyrophosphate (PPi), tripolyphosphate (polyP3) or inositol polyphosphates (inositol hexakisphosphate, IP6; 5-diphosphoinositol pentakisphosphate, IP7; and 5-diphosphoinositol tetraphosphate, PP-IP4) resulted in sodium-dependent depolarization of the oocyte membrane potential and increase in Pi conductance. Deletion of their SPX domains abolished this stimulation. Inositol polyphosphates generated outward currents in Pi/Na+ -loaded giant vacuoles prepared from wild type yeast or from pho91? yeasts expressing TbPho91 but not from pho91?, or from yeasts expressing SPX domain mutants of PHO91, or TbPho91. Our whole vacuole patch clamp results indicate that TbPho91 and Pho91p are responsible for vacuolar Pi and Na+ efflux and that inositol polyphosphate derivatives regulate the Na+/Pi symporters through their SPX domains.

FT3: A bacterial complex is required for plastid integrity in P. falciparum

Florentin, Anat (University of Georgia); *Stephens, Dylon (University of Georgia); Muralidharan, Vasant (University of Georgia)*

The deadly malaria parasite, Plasmodium falciparum, contains a non-photosynthetic plastid known as the apicoplast, that functions to produce essential metabolites. Little is known about its biology or regulation, but drugs that target the apicoplast are clinically effective. Previous studies have identified several prokaryotic Clp (caseinolytic protease) genes, encoded by the Plasmodium genome. In bacteria, the evolutionary ancestors of the apicoplast, and in plants chloroplasts these proteins form complexes that degrade proteins in a proteasome-like manner to regulate key cellular processes, but their function in the apicoplast is completely unknown. Using an array of molecular tools, we genetically targeted members of the apicoplast Clp complex and generated conditional mutants of the apicoplast-localized PfClpC chaperone, PfClpP protease, and PfClpR, an inactive protease subunit. Using these genetic tools, we showed that these genes are essential for parasite viability through their central role in maintaining a functional apicoplast. Inactivation of Clp family members leads to loss of the entire organelle and can be rescued by addition of the essential apicoplast-derived metabolite, IPP. We further demonstrated physical and genetic interactions between the Clp proteins, revealing a unique mode of regulation of Clp complex activity. We designed a genetic reporter for Clp activity to study the complex ability to degrade substrates in vivo. Finally, we are using ClpP conditional mutants to screen for antimalarial compounds that target the clp complex. These data demonstrate the essential function of Clp family members in maintaining apicoplast integrity and their potential roles as antimalarials.

FT4: A large conductance ion channel on the malaria parasite digestive vacuole

Saggu, Gagandeep S. (LMVR, NIAID, National Institutes of Health)*; Desai, Sanjay (LMVR, NIAID, National Institutes of Health)*

The malaria parasite digestive vacuole (DV) serves an essential role in hemoglobin digestion, is the target of several antimalarial drugs including chloroquine, and is thought to resemble lysosomes of higher organisms. The DV is presumed to have multiple transmembrane channels and pumps to that maintain an acidic internal pH, export amino acids, and mediate flux of antimalarial drugs. Study of these transporters has been limited to macroscopic flux measurements on small organelles; these studies have required indirect transport assays and have been further complicated by heterogeneity of cellular preparations. We have now addressed these limitations with cell-attached patch-clamp of individual, intact DV membranes from Plasmodium falciparum. High resistance seals (> 10 Gohms) were obtained after optimizing pipette glass composition and geometry. A large-conductance channel (450 pS in 320 mosm salt solutions) was the primary channel seen, but additional smaller conductance channels have also been detected. The primary channel exhibited weak voltage-dependence at large imposed membrane potentials as well as unusual gating that suggests a two-pore configuration on the DV membrane. Progress on identification of this channel's molecular basis and the effects of antimalarial drugs and nutrients on channel activity will also be described. These findings indicate that the DV membrane has robust transport activity with features distinct from those of the mammalian lysosome. Identification and molecular characterization of transporters on the DV membrane will provide foundational insights into vacuolar biology, clarify the resistance mechanisms for several antimalarial drugs, and guide development of new therapies for malaria.

TT1: CATHEPSIN L (TBCATL) PROCESSING AND POST-GOLGI SORTING TO THE LYSOSOME IN AFRICAN TRYPANOSOMES

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

Cathepsin L (TbCatL), a lysosomal cysteine protease, is a critical virulence factor in the bloodstream-stage of Trypanosoma brucei. TbCatL is synthesized as two precursor forms (full-length P and smaller X of uncertain identity) with an N-terminal prodomain that mediates post-Golgi sorting, and which is removed upon arrival in the lysosome to generate mature enzyme (M). Post-Golgi sorting of TbCatL to the lysosome in procyclic parasites is clathrin-mediated. To examine TbCatL trafficking we created a procyclic cell line ectopically expressing a truncated TbCatL reporter without the non-catalytic C-terminal domain (CTD) (TbCatLΔ) in an RNAi cell line targeting the CTD and 3'UTR of the endogenous mRNA. TbCatL silencing is lethal and is rescued by TbCatLΔ. TbCatLΔ localizes properly to the lysosome and its processing recapitulates endogenous TbCatL activation (P' & X' → M'). X' forms with ER-retained TbCatLΔ:MDDL, but not with catalytically inactive TbCatLΔ:C150A, indicating that it is generated by cis-catalysis in the ER of newly synthesized P' within the prodomain. TbCatL modeled on the known structure of human CatL suggests three solvent accessible features that could contain post-Golgi targeting signals: the N-terminus, an α-helix:turn junction (α1-t1), and a separate turn (t3). Mutation of E34/E35 in the N-terminus had no effect on trafficking. K47, G49, and K50 of the α1-t1 region had previously been implicated in trafficking of the T. cruzi orthologue [Huete-Perez et al. (1999) JBC 274:16249], but we found no such effect in T. brucei. However, mutation of N79/P80 in t3, conserved in all kinetoplastids, resulted in impaired lysosomal trafficking and elevated secretion. Collectively these results indicate that N79/P80 are the post-Golgi sorting signal for CatL in African trypanosomes.

TT2: Characterization of vesicular systems of artemisinin resistance suggest their roles in trafficking and virulence, dependent and independent of Kelch13

Suresh, Niraja (University of Notre Dame); *Khair, Maisha; Coppens, Isabelle; Bhattacharjee, Souvik; Mbengue, Alassane; Ghorbal, Mehdi; Haldar, Kasturi*

The identification of P. falciparum Kelch13 (K13) as a major marker of artemisinin resistance has significantly aided underlying mechanisms of resistance. We have previously shown that major resistance mutation K13C580Y antagonizes its binding to PfPI3K) binds K13, increasing levels of the kinase and its lipid product PI3P, to confer resistance. K13 localizes to vesicles enriched in PI3P and whose amplification is sufficient to induce the parasite unfolded protein response (UPR) associated with in vivo clinical resistance (Mok et al. 2015) as well as resistance independent of K13.

Cryoimmunoelectron microscopy reveals that K13 vesicles emerge from membranes enriched in BiP, the major chaperone marker and mediator of the UPR in the ER. Modeling of quantitative proteomic and transcriptomic data combined with high resolution microscopy separates how K13 vesicles engage parasite ER-Golgi machinery of constitutive secretion as well as that host targeted export (HT/PEXEL and PNEP proteins) that remodel the host erythrocyte. The findings advance K13-dependent and -independent vesicular mechanisms of resistance and how resistant organisms unexpectedly exploit virulence mechanisms of endothelial engagement that promote persistence in host tissues.

Session VIII: Clubbed to Death

Chairs: Jennifer Guler and Selina Bopp

Wednesday, September 12, 2018
9:00 am – 11:30 am

FT1: TgBDP3 is a member of the Toxoplasma TFIID complex and a target of the anti-parasitic bromodomain inhibitor I-BET151

Jeffers, Vicki (Indiana University School of Medicine); *Hanquier, Jocelyne (Indiana University School of Medicine); Sullivan Jr, William J. (Indiana University School of Medicine)*

The acetylation of histone tails is a critical process for the regulation of gene expression in Toxoplasma gondii. The bromodomain is the recognition module for acetylated lysines and bromodomain-containing proteins are recruited to acetylated histone tails to coordinate transcriptional regulatory complexes. There are twelve bromodomain-containing proteins in Toxoplasma, only two of which (the GCN5 lysine acetyltransferases) have been characterized. We have previously demonstrated that the bromodomain inhibitor I-BET151 has selective anti-parasitic activity against Toxoplasma; here, we present findings that elucidate a likely target of I-BET151. Inspection of the dozen bromodomain-containing proteins in Toxoplasma reveals that TGME49_258990 (TgBDP3) is the only one that contains two tandem N-terminal bromodomains, a domain architecture resembling the targets of I-BET151 in humans. We demonstrate that TgBDP3 is an essential nuclear protein that is associated with the TFIID transcriptional initiation complex. Using chemoproteomics approaches with a linkable I-BET analogue, we have determined that I-BET151 binds the first (but not the second) bromodomain of TgBDP3. Chromatin-immunoprecipitation studies are underway to identify the gene network that is regulated by TgBDP3, and an inducible tgbdp3 knock-down will uncover the essential biological role of TgBDP3 in tachyzoite viability.

FT2: Delayed death in the malaria parasite: prenylation dependant disruption of intracellular trafficking

Kennedy, Kit (Department of Biochemistry and Molecular Biology, The University of Melbourne)*; Cobbold, Simon (Department of Biochemistry and Molecular Biology, The University of Melbourne); Spillman, Natalie (School of Biosciences, The University of Melbourne); Hanssen, Eric (Advanced Microscopy Facility, Bio21 Molecular Science and Biotechnology Institute); Blanch, Adam (Department of Biochemistry and Molecular Biology, The University of Melbourne); Namvar, Arman (Department of Biochemistry and Molecular Biology, The University of Melbourne); Tilley, Leann (Department of Biochemistry and Molecular Biology, The University of Melbourne); McConville, Malcolm (Department of Biochemistry and Molecular Biology, The University of Melbourne); Ralph, Stuart (Department of Biochemistry and Molecular Biology, The University of Melbourne)*

Malaria parasites possess a 'plastid-like' organelle called the apicoplast. Translation inhibitors that selectively target the apicoplast are lethal for the parasite. Several such compounds are clinically used as antimalarials, including doxycycline and clindamycin. However, parasites treated with an apicoplast inhibitor only arrest in their subsequent intra-erythrocytic development cycle, a characteristic known as the 'delayed death' effect.Treated parasites replicate once but transmit a defective apicoplast to their daughter cells. These progeny consequently cannot produce the sole essential metabolic product of the apicoplast in asexual blood stages: the isoprenoid precursor isopentenyl-pyrophosphate. However, it remains unclear what consequences isoprenoid depletion has on parasite cellular processes and how they contribute to parasite death. We have investigated the essentiality of isoprenoid compounds and characterised the molecular and morphological phenotype of delayed death. Metabolomic analysis together with fluorescence uptake experiments suggest that there is a disruption in digestive vacuole (DV) function and biogenesis in parasites undergoing delayed death. This is further supported by our analysis using serial block-face scanning electron microscopy, which shows that the DV fragments into multiple compartments in delayed death parasites and that these parasites also have abnormal uptake of the erythrocyte cytosol. We hypothesise that these phenomena arise from a defect in trafficking to the DV due to aberrant prenylation of vesicular trafficking proteins. Supplementing delayed death parasites with geranylgeraniol, an isoprenoid precursor used for protein prenylation, fully reverses the abnormal DV phenotype and temporarily rescues parasites for an additional infection cycle. This suggests that interruption of protein prenylation, and consequent cellular trafficking defects, are the proximal causes of delayed death.

FT3: Antimalarial N-Myristoyl Transferase inhibitors: a resistant mutant parasite provides insights into inhibitor binding mode

Schlott, Anja (Francis Crick Institute); *Mayclin, Stephen (Beryllium Discovery, Centre for Infectious Disease Research Seattle); Reers, Alexandra (Centre for Infectious Disease Research Seattle Structural Genomics Center for Infectious Disease); Coburn-Flynn, Olivia (Columbia University Medical Center); Bell, Andrew (Imperial College London); Green, Judith (The Francis Crick Institute); Knuepfer, Ellen (The Francis Crick Institute); Charter, David (GlaxoSmithKline); Lyons-Abbott, Sally (Centre for Infectious Disease Research, Seattle Structural Genomics Center for Infectious Disease); Staker, Bart (Centre for Infectious Disease Research, Seattle Structural Genomics Center for Infectious Disease); Myler, Peter (Centre for Infectious Disease Research, Seattle Structural Genomics Center for Infectious Disease); Chung, Chun-wa (GlaxoSmithKline); Fidock, David (Columbia University Medical Center); Tate, Edward (Imperial College London); Holder, Anthony (The Francis Crick Institute)*

Protein N-myristoylation is the attachment of the 14-carbon fatty acid myristate onto the N-terminal glycine of certain proteins. This largely co-translational modification is catalysed by N-myristoyltransferase (NMT) and is involved in localizing the substrate protein to specific membranes. In Plasmodium falciparum, NMT substrates are involved in a variety of essential processes including host cell invasion, parasite gliding motility, protein trafficking and protein degradation. NMT has been validated as a therapeutic target in numerous parasitic diseases including malaria. Treatment with an NMT inhibitor results in rapid reduction of P. falciparum growth in vitro and invivo using a SCID mouse model. We have used crystallography to study the structural basis of potent NMT inhibitors selective for Plasmodiumspp. with the enzyme. Parasites resistant to an entire inhibitor series were generated and we identified that resistance is mediated by a single amino acid substitution in the substrate binding pocket. The binding of another drug series remains unaffected by this mutation. We successfully manipulated P. falciparum using CRISPR-Cas9 to confirm that the G386E mutation selected under drug pressure was causal for in vitro resistance. We tagged myristoylated proteins expressed in the mutant parasite via a copper catalysed cycloaddition reaction and multifunctional probes. We then introduced the same amino acid into recombinant Plasmodium vivax NMT, which can be easily expressed and purified. In vitro parasite growth-, enzyme activity and SPR assays with structurally different NMT inhibitors provided insight into the different drug binding modes. This approach, to incorporate the study of resistance early-on in the drug development process, will help formulation of solutions to bypass evolution of parasite drug resistance.

TT1: Mutations in the actin-binding protein PfCoronin confer resistance to Artemisinin in West African Plasmodium falciparum isolates

Sharma, Aabha (Harvard T. H. Chan School of Public Health)

Efforts to control malaria will be in serious jeopardy if an emerging drug resistance to Artemisinin (ART) in Southeast Asia spreads to the African continent, which bears the highest global malaria burden. Therefore, we chose two Senegalese field isolates of Plasmodium falciparum to investigate ART resistance. These isolates are susceptible to ART treatment and do not have mutations in the propeller domain of PfKelch13, a known marker of ART resistance. After intermittent and step-wise dihydroartemisinin (DHA) pulses started in 2011 and continued over four years, we obtained three independent ART resistant lines, all of which had a significant increase in their 0-3hrRing Stage Survival Assay (RSA) survival percentage (6%, 7.9% and 9.6% respectively) compared to their parents (0-3hrRSA<1%). They had no change in EC50 response to ART derivatives in a 72-hour dose-response assay.

Whole genome sequencing of ART resistant lines identified thirteen different single nucleotide polymorphisms (SNPs) in ten genes, which did not include PfKelch13. PfCoronin (PF3D7_1251200), which codes for a protein similar in structure to PfKelch13, had new SNPs in its WD-40 propeller domain of all three resistant lines. We generated CRISPR-Cas9 edited parasites containing PfCoronin SNPs (G50E, R100K, E107V) in their Senegalese parental background. RSA survival percentage from two independent clones for each background showed Coronin SNPs to be sufficient to confer ART resistance (6.7% and 9.4% in Background 1 and 5.2% and 5.3% in Background 2) compared to their parents (0-3hrRSA<1%). This suggests that PfCoronin is a determinant of ART resistance in West African parasites. Further investigation of biological mechanism of ART resistance conferred by PfCoronin will make significant contributions to the current understanding of ART resistance.

TT2: Mind the traffic: a role for trafficking in the cell stress response and artemisinin resistance in P. falciparum

Henrici, Ryan C. (London School of Hygiene & Tropical Medicine); *Zoltner, Martin; Hart, Melissa; Edwards, Rachel; van Schalkwyk, Don; Baker, David; Moon, Rob; Odom John, Audrey; Field, Mark; Sutherland, Colin*

Artemisinin susceptibility in Plasmodium falciparum is modulated by mutations in the gene pfkelch13, which encodes a kelch propeller domain protein of unknown function. Reduced susceptibility is demonstrated in vitro by elevated parasite survival after short exposures to a physiologic concentration of drug in the early ring stage. Using CRISPR-Cas9 genome editing, we provide the first evidence of a similar but K13-independent in vitro artemisinin resistance caused by a single base change in the AP-2 adaptor protein complex mu-subunit (pfap2µ). Our functional characterisation of PfAP2µ validates that the gene encodes a clathrin-independent, non-canonical AP-2 trafficking factor that colocalises with K13 in the cytoplasm and is essential for multiple organelle biogenesis and asexual replication. We show that disruption of this traffic in early rings induces the cell stress response that underlies artemisinin resistance and induced dormancy. A model depicting a role for intracellular traffic in ring-stage artemisinin resistance is proposed.

TT3: Characterizing known translation inhibitors and drug candidates via Plasmodium falciparum whole cell extracts vs. S35 methionine incorporation: separating true 80S ribosome inhibition from artifact

Sheridan, Christine*; Garcia, Valentina; Ahyong, Vida; DeRisi, Joseph*

FT4: Development of drug-resistant Naegleria fowleri to identify target of novel class of amidines

Colon, Beatrice L. (University of Georgia)*; de Paula Baptista, Rodrigo (University of Georgia); Farahat, Abdelbasset (Georgia State University); Boykin, David (Georgia State University); Kyle, Dennis (University of Georgia)*

The brain-eating amoeba, Naegleria fowleri, is a free-living amoeba that causes the disease primary amoebic meningoencephalitis (PAM). The amoeba can infect healthy individuals and within 2 weeks cause nearly 97% fatality.

Although there have been 3 survivors in recent years, current therapeutics are sub-optimal and current drug discovery efforts are minimal. Amidines and diamidines have been previously used for infectious diseases particularly with the free-living amoeba. Our previous work identified 6 amidino structural classes that showed inhibition of N. fowleri. With additional characterization and optimization of these compounds, we were able to identify nanomolar potent activity that inhibit the amoeba within the first 8 hours of drug exposure. The purpose of this study was to induce resistance to elucidate a potential target for a class of active amoebicidal compounds. We created resistance in N. fowleri to DB2518, a fluorescent amidine, by step-wise increase of constant drug pressure in culture. We found the resistant line tolerates approximately 30 times higher concentration of drug than our sensitive line. Additionally, with fluorescent microscopy we have identified morphological differences between the resistant and sensitive lines, specifically size and number of vacuoles.

Moreover, we found the resistant amoeba also have a degree of tolerance to compounds within the same structural class. Lastly, we are analyzing whole-genome sequence data for clones from the resistant and sensitive lines to identify any genomic mutations that could elucidate a potential mechanism of action of DB2518.

FT5: Unravelling chromosomal segregation in Trypanosomatid parasites: the nucleoporin TbMLP1 plays an essential role in the maintenance of ploidy

Sterkers, Yvon (UMR MIVEGEC (CNRS 5290 - IRD 224 - Université Montpellier) CHU de Montpellier)*; Yagoubat, Akila (UMR MIVEGEC (CNRS 5290 -IRD 224-Université Montpellier)); Stanojcic, Slavica (UMR MIVEGEC (CNRS 5290 -IRD 224 - Université Montpellier)); Berry, Laurence (Microscopie Electronique Analytique Facility, Montpellier, France); Bryant, Jessica (Unité Biologie des Interactions Hôte-Parasite, Dép. de Parasites et Insectes Vecteurs, Institut Pasteur, CNRS, ERL 9195, 75015 Paris); Crobu, Lucien (UMR MIVEGEC (CNRS 5290 - IRD 224 - Université Montpellier)); Kuk, Nada (UMR MIVEGEC (CNRS 5290 - IRD 224 - Université Montpellier)); Bastien, Patrick (UMR MIVEGEC (CNRS 5290 - IRD 224 - Université Montpellier) Chu de Montpellier); Scherf, Artur (Unité Biologie des Interactions Hôte-Parasite, Département de Parasites et Insectes Vecteurs, Institut Pasteur, CNRS, ERL 9195, 75015 Paris)*

Trypanosomatids are a group of divergent eukaryotes of medical and economical relevance that include Trypanosoma spp and Leishmania spp. These early branching eukaryotes are characterized by molecular and cellular features such as marked aneuploidy in Leishmania species. Nuclear pore complexes (NPCs) are large multiprotein complexes embedded in the nuclear envelope of eukaryotic cells. The primary function of NPCs is to regulate nucleo-cytoplasmic transport; however, recent research indicates that nucleoporins (NUPs), which are components of NPCs, are emerging as an important regulator in other core processes including chromosomal segregation, transcription, and cytokinesis. Twenty-two NUPs, including two Myosin-like proteins (MLP), were identified in the Trypanosomatids. Here, RNAi knockdown of the nucleoporin TbMLP1 led to a severe growth reduction of T. brucei procyclic forms associated with abnormal NucleusKinetoplast pattern and aneuploidy verified by FISH and flow cytometry. Two days post induction of knockdown, less than 50% of cells remained disomic. As soon as 8 hours post induction, 70% of cells divided asymmetrically, and the total number of homologs was odd (i.e: 1:2 and 2:3) in >97% of recorded dividing nuclei, suggesting a defect in the regulation of replication. Using CRISPR-Cas9 for in situ tagging, we confirmed by immunofluorescence and immunoelectron microscopy that MLP1 localizes at the nuclear basket of nuclear pores in T. brucei and Leishmania, as it is the case in yeast. Taken together, our results indicate that TbMLP1 is required for the maintenance of diploidy in T. brucei. Our data provide new insights into the molecular mechanisms regulating genome integrity and its interesting connection to the NPCs in Trypanosomatids and point to a molecular process that may contribute to Leishmania aneuploidy.

FT6: Investigation of arginine methylation function in Leishmania (V.) braziliensis growth and infection

Lorenzon, Lucas (University of São Paulo)*; Diniz, Juliana (University of São Paulo); Ferreira, Tiago (University of York); Walrad, Pegine (University of York); Cruz, Angela (University of São Paulo)*

Leishmania (V.) braziliensis is the predominant cause of cutaneous and mucocutaneous leishmaniasis in Brazil. In trypanosomatids, control of gene expression occurs mainly at the post-transcriptional level, and RNA binding proteins (RBPs) are key players in the determination of transcript fate. Among other post-translational modifiers, RBPs are targets of Arginine Methyltransferases (PRMTs), which transfer a methyl group to arginine residues of proteins, affecting their RNA-binding capacities. Herein we present the preliminary characterization of the five predicted arginine methyltransferases of L. (V.) braziliensis. The comparative immunoblotting analysis of the arginine methylation profile of L. (V.) braziliensis between procyclic promastigotes, metacyclics and amastigotes reveal a diverse profile of arginine methylation in the different stages, as well as modulation of expression of the five putative PRMTs. Overexpression (OE) and knockout (KO) of these genes led to changes in parasite protein methylation profile.

Based on the KO and OE phenotypic analyses, PRMTs are not essential genes for L. (V.) braziliensis promastigote survival, at least independently, and investigation of candidate targets is in progress. Although no differences were observed in axenic promastigote growth for any of the transfectants when compared to wild-type cells, macrophage infection by PRMT5 OE parasites is significantly lower than by other cell lines. According to preliminary data, PRMT5 is the only PRMT which is expressed both in nucleus and cytoplasm in L. (V.) braziliensis, which may suggest divergent functions. Overall, the data indicate that these PRMTs promote methylation of a diverse group of proteins in different stages of the parasite life cycle and the relevance of the post-translational modification for the nucleic-acid binding activities, gene expression regulation, and virulence will be investigated. Financial support: FAPESP, CNPq and CAPES.

TT4: Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi

Berná, Luisa (Institut Pasteur de Montevideo); *Rodriguez, Matias; Chiribao, Maria Laura; Parodi-Talice, Adriana; Pita, Sebastián; Rijo, Gastón; Alvarez-Valin, Fernando; Robello, Carlos*

Although the genome of Trypanosoma cruzi, the causative agent of Chagas disease, was first made available in 2005, with additional strains reported later, the intrinsic genome complexity of this parasite (the abundance of repetitive sequences and genes organized in tandem) has traditionally hindered high-quality genome assembly and annotation. This also limits diverse types of analyses that require high degrees of precision. Long reads generated by third-generation sequencing technologies are particularly suitable to address the challenges associated with T. cruzi's genome since they permit direct determination of the full sequence of large clusters of repetitive sequences without collapsing them. This, in turn, not only allows accurate estimation of gene copy numbers but also circumvents assembly fragmentation. Here, we present the analysis of the genome sequences of two T. cruzi clones: the hybrid TCC (TcVI) and the non-hybrid Dm28c (TcI), determined by PacBio Single Molecular Real-Time (SMRT) technology. The improved assemblies herein obtained permitted us to accurately estimate gene copy numbers, abundance and distribution of repetitive sequences (including satellites and retroelements). We found that the genome of T. cruzi is composed of a 'core compartment' and a 'disruptive compartment' which exhibit opposite GC content and gene composition. Novel tandem and dispersed repetitive sequences were identified, including some located inside coding sequences. Additionally, homologous chromosomes were separately assembled, allowing us to retrieve haplotypes as separate contigs instead of a unique mosaic sequence. Finally, manual annotation of surface multigene families, mucins and trans-sialidases allows now a better overview of these complex groups of genes.

TT5: Functional characterization of mitochondrial translation components in the early diverging eukaryote Toxoplasma gondii

Lacombe, Alice (Wellcome Centre for Molecular Parasitology, University of Glasgow); *J. Tottey1, J. Ovciarikova1, F. Courjol2, M. Gissot2 , L. Sheiner1 1- Wellcome Centre for Molecular Parasitology, University of Glasgow 2- University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019*

Apicomplexan parasites are divergent organisms whose cellular machineries often consist of unique structures of functions compared to popular model organisms. Studying fundamental mitochondrial biology in these organisms means defining the ancestral core of eukaryotic pathways while simultaneously identifying organism specific traits, that may also inspire drug discovery. Organelle translation has been a focus for the latter in recent years. Due to extreme gene transfer to the nuclear genome, the apicomplexan mitochondrial genome encodes only three proteins: COXI, COXIII and COB, and a series of indirect evidence suggest active mitochondrial translation of these proteins. Evidence also point to several divergent features in this pathway compare to model organism, primarily the reliance on the import of the full set of tRNAs from the cytosol, and the unusual ribosome composition. We investigate the molecular detail of translation in apicomplexan using Toxoplasma gondii as a model. Our bioinformatics screen, based on gene expression patterns, identified candidate for novel players in mitochondrial translation in T. gondii. Gene tagging and immunofluorescence confirmed the mitochondrial location of 12 out of 15 proteins tested and conditional knockdown of each of three of these genes points to their importance for growth. This group included the T. gondii homolog of the mitochondrial ribosomal S35 subunit, validating our search. S35 depletion result in impairs mitochondrial functions such as protein import. Pull down experiment with biotinylated tRNAs identified further candidate protein for control of mitochondrial translation, and provided evidence for a mitochondrion – nucleus interaction that may facilitate tRNA exchange. Our findings provide further support for the divergent nature of apicomplexan mitochondrial translation and highlight new proteins involved in its control in T. gondii.

TT6: Antimalarial Drug Target PfATP4 is Present in Parasite Plasma Membrane as a Large Complex

Ramanathan, Aarti*; Vaidya, Akhil; Bhatnagar, Suyash; Morrisey, Joanne*

Plasmodium falciparum P-type cation ATPase (pfATP4) is a Na+/H+ efflux pump crucial for maintaining low cytosolic Na+ concentration in malaria parasites during its intraerythrocytic lifecycle. In recent years, pfATP4 has been the subject of significant interest since a range of mutations in this protein confer resistance to multiple classes of new generation of antimalarials, including the spiroindolones (KAE609), pyrazoleamides and dihydroisoquinolones (SJ733). Influx of Na+ into the parasite due to pfATP4 inhibition is considered to be the common mechanism of action of these drugs. In addition, screening of MMV's 'Malaria box' identified 28 compounds belonging to 17 different chemical classes targeting Na+ homeostasis. Thus, it is important to understand the pathways affected by Na+ homeostasis disruption that result in parasite demise. We have previously shown that a short exposure (2 hours) to pyrazoleamide and spiroindolones result in dramatic alterations in parasite morphology resembling a premature schizogony. We also found that the influx of Na+ results in inhibition of an active process of cholesterol exclusion from the parasite plasma membrane which renders the drug treated parasites sensitive to saponin. Understanding the regulation of pfATP4 is crucial for understanding the biology of Na+ signaling in malaria parasites. Characterization of pfATP4 via by blue-native page analysis revealed that it exists in the parasite plasma membrane as a large complex. Currently, experiments are underway for proteomic characterization of components of the complex as well as reconstitution of the transporter in lipid bilayer.

Session IX: Switch It On, Switch Off

Chairs: Galadriel Hovel-Minor and Sumiti Vinayak

Thursday, September 13, 2018

9:00 am – 10:00 am

FT1: Role of the eIF4E1-4EIP complex in gene repression during differentiation of trypanosomes

Marucha, Kevin (ZMBH)*; Egler, Franziska (ZMBH); Terrao, Monica (ZMBH); Mugo, Elisha (ZMBH); Braun, Johanna (ZMBH); Droll, Dorothea (ZMBH); Minia, Igor (ZMBH); Clayton, Christine (ZMBH)*

Trypanosomes rely on post-transcriptional mechanism to regulate gene expression. They have six eIF4Es of varying cap-binding affinities and five eIF4Gs, suggesting multiple possibilities for translation regulation. Trypanosome eIF4E1 does not interact with any of the eIF4Gs but instead interacts with 4E interacting protein - 4EIP. Tethering of either eIF4E1 or 4EIP to a reporter mRNA results in suppressed expression. A truncated 4EIP lacking the canonical eIF4E-binding motif remains suppressive, but suppression by eIF4E1 requires 4EIP. Bloodstream form Trypanosoma brucei lacking either 4EIP or eIF4E1 have only a mild growth defect. At high parasitemia, bloodstream forms decrease translation and become growth-arrested stumpy forms, which are primed to differentiate to procyclic forms when taken up by a tsetse fly. Interestingly, lack of 4EIP compromises this, and the defect can be rescued by the truncated 4EIP that in unable to bind eIF4E1. 4EIP null stumpy forms have sustained high protein synthesis rates indicating that 4EIP is required for translation suppression during differentiation to the stumpy form. In contrast bloodstream forms lacking eIF4E1 become stumpy but are unable to convert to growing procyclic forms. In line with its repressive functions, 4EIP in bloodstream forms is preferentially associated with unstable mRNAs. We here suggest that 4EIP and EIF4E1 fine-tune mRNA levels in growing cells, and that 4EIP has functions which are independent of EIF4E1.

FT2: Extensive m6A RNA methylation dynamically modulates gene expression in Plasmodium falciparum

Baumgarten, Sebastian (Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris)*; Bryant, Jessica (Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris); Sinha, Ameya (School of Biological Sciences, Nanyang Technological University, Singapore); Reyser, Thibaud (Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris); Dedon, Peter (Department of Biological Engineering, Massachusetts Institute of Technology, USA); Preiser, Peter (School of Biological Sciences, Nanyang Technological University, Singapore); Scherf, Artur (Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris)*

Malaria pathogenesis results from the massive asexual replication of Plasmodium falciparum within human red blood cells, a process that is based on a precisely timed periodic cascade of gene expression. However, the mechanism by which such a hardwired transcriptional program is fine-tuned at the protein expression level remains largely unknown. Chemical modifications on mRNA and especially the prevalent N6 methyladenosine (m6A) are emerging as a regulator of RNA homeostasis in higher eukaryotes. We determined global mRNA modifications by mass-spectrometry in P. falciparum asexual blood stage development and identified m6A to be by far the most abundant and dynamically regulated modification. Surprisingly, it is present at up to 5-fold higher levels than in any other eukaryotic organism studied as yet, suggesting a prominent role for m6A in posttranscriptional regulation. We further demonstrated by parallel m6A immunoprecipitation and RNA sequencing that individual m6A sites are highly differentially methylated during the parasite's development. Using CRISPR interference, we characterized the RNA methyltransferase PfMT-A70 as an evolutionarily conserved, integral member of the m6A writer complex. Disruption of m6A methylation leads to increased levels of transcripts that carry this modification in wild type parasites. Correspondingly, we found that m6A methylation inversely correlates with mRNA half-life as well as translational efficiencies. Collectively, this study demonstrates the regulatory potential of extensive m6A mRNA methylation in fine-tuning an inherently dynamic transcriptional program and adds a new 'epitranscriptomic' player to gene regulation in malaria parasites.

FT3: Investigation of the Not1 proteins in regulating gene expression in Plasmodium falciparum

Liu, Ying (Nanyang Technological University)*; Featherstone, Mark; Bozdech, Zbynek (Nanyang Technological University)*

Plasmodium falciparum is the most deadly form of malaria that threatened millions of people around the world. The CCR4-NOT complex is a highly conserved complex that regulates gene expression across the eukaryotic kingdom and Not1 is the scaffold of the complex [1]. P. falciparum encodes two homologs of Not1, Not1.1 (PF3D7_1103800) and Not1.2 (PF3D7_1417200).

My study investigates the function of two Not1 proteins in gene regulation in P. falciparum during 48-hour intraerythrocytic developmental cycle (IDC). The transcript levels of 235 genes were significantly changed in not1.1 knockout. Multiple invasion-related genes were downregulated in late stages of IDC, possibly leading to the defective growth and invasion rate. Besides, the upregulation of rifins and KAHRP gene suggests that Not1.1 plays a role as parasite virulence regulator. Moreover, not1.1 knockout affects the recruitment of elongating RNA Polymerase II globally but the changes in transcriptional initiation are not significantly correlated to the transcript changes. Interestingly, most transcripts with significant changes in not1.1 knockout are positively correlated with those in caf1 mutant [2], indicating that Not1.1 might be required for the function of Caf1 subunit. On the other hand, not1.2 knockdown displayed slower growth rate and fewer transcripts were changed to less extents. Strikingly, these transcripts were oppositely affected in not1.1 knockout. In conclusion, Not1.1 regulates gene expression through post-transcriptional regulation by affecting Caf1. Two homologs of Not1 genes perform antagonistic functions in P. falciparum.1. Collart, M.A. and O.O. Panasenko, The Ccr4–Not complex. Gene, 2012. 492(1): p. 42-53.2. Balu, B., et al., CCR4-Associated Factor 1 Coordinates the Expression of Plasmodium falciparum Egress and Invasion Proteins. Eukaryotic Cell, 2011. 10(9): p. 1257-1263.

FT4: Identification of PfAP2-HS as the master regulator of the heat shock response in Plasmodium falciparum

***Tintó-Font, Elisabet (ISGlobal)**; Michel-Todó, Lucas (ISGlobal); Cortés, Alfred (ISGlobal, ICREA)*

Cyclical febrile episodes are one of the most common symptoms of Plasmodium falciparum infections, reaching temperatures of up to 41ºC during 2-6h. We have previously shown that different parasite lines vary in their survival to heat shock simulating febrile temperatures, but the mechanism underlying heat shock resistance remains unknown. Full genome sequencing revealed a mutation present only in heat shock-sensitive parasite clones that results in a premature STOP codon in the gene PF3D7_1342900 (here named pfap2-hs), which encodes an ApiAP2 transcription factor. Wild-type parasites showed a massive increase in hsp70-1 transcript levels during heat shock, whereas this response was delayed and of much lower magnitude in parasites with truncated PfAP2-HS. Using CRISPR/Cas9 technology we generated pfap2-hs knock-out parasites that, in addition to presenting a heat shock-sensitive phenotype, showed a severely reduced growth at 37.5ºC or 37ºC, but not at 35ºC. Moreover, a delayed cell cycle and a reduced number of merozoites per schizont were observed in this knock-out parasite line compared to wild-type parasites, implying that PfAP2-HS plays a role in normal asexual development. Microarray analysis corroborated the absence of an immediate hsp70-1 and hsp86 response in sensitive parasites and characterized the initial PfAP2-HS-dependent transcriptional response to heat shock. ATAC-seq analysis of wild-type and pfap2-hs knock-out parasites is underway to identify the DNA binding sites of this transcription factor.

Altogether, our results show that the PfAP2-HS transcription factor plays an essential role as the master regulator of the heat shock response, providing the first clear identification of a transcriptional regulator that drives a protective response in front of external changes in P. falciparum blood stages.

Session X: The best for last

Chairs: Sumiti Vinayak and Galadriel Hovel-Minor

Thursday, September 13, 2018

10:30 am – 11:30 am

FT5: Structural studies of a novel SET-domain containing lysine methyltransferase that regulates motility of Toxoplasma gondii

Pivovarova, Yulia (Medical University of Vienna); *Lesigang, Johannes (Medical University of Vienna); Hu, Ke (Indiana University); Liu, Jun (Indiana University); Dong, Gang (Medical University of Vienna)*

Lysine methyltransferases (KMTs) were initially associated with transcriptional control through their methylation of histones and other nuclear proteins, but have since been found to regulate many other cellular activities. The apical complex lysine (K) methyltransferase (AKMT) of the human parasite Toxoplasma gondii was recently shown to play a critical role in regulating cellular motility. Here we report a 2.1-Å resolution crystal structure of the conserved and functional C-terminal portion (aa289-709) of T. gondii AKMT. AKMT dimerizes via a unique intermolecular interface mediated by the C-terminal TPR (tetratricopeptide repeat)-like domain together with a specific zinc-binding motif that is absent from all other KMTs. Disruption of AKMT dimerization impaired both its enzyme activity and parasite egress from infected host cells in vivo. Structural comparisons reveal that AKMT is related to the KMTs in the SMYD family, with, however, a number of distinct structural features in addition to the unusual dimerization interface. These features are conserved among the apicomplexan parasites and their free-living relatives, but not found in any known KMTs in animals. AKMT therefore is the founding member of a new subclass of KMT that has important implications for the evolution of the apicomplexans.

FT6: Evolution of mitochondrial TAT translocases illustrates the demise of bacterial protein transport machines in mitochondria

Markéta, Petru (Department of Parasitology, Faculty of Science, Charles University, Prumyslová 595, Vestec, 252 42, Czech Republic); *Wideman, Jeremy (Wissenschaftskolleg zu Berlin, Wallotstrasse 19, 14193, Berlin, Germany); Moore, Kristoffer (Division of Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK); Alcock, Felicity (Department of Biochemistry, University of Oxford, Oxford, United Kingdom); Palmer, Tracy (Division of Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK); Doležal, Pavel (Department of Parasitology, Faculty of Science, Charles University, Prumyslová 595, Vestec, 252 42, Czech Republic)*

In the past, ancestor of eukaryotic cell adopted alphaproteobacteria, which subsequently became mitochondrion. During the development of endosymbiosis, the protein transport between the host and the endosymbiont had to be evolved. "Classical" aerobic mitochondria contain components of bacterial origin (TOM and SAM complex of the outer mitochondrial membrane, Oxa1 in the inner mitochondrial membrane) and eukaryotic innovations such as TIM complex in the inner mitochondrial membrane. The last eukaryotic common ancestor (LECA) probably already contained these four main protein translocation machines. Special group of eukaryotes carries additional bacterial genes for protein transport – SecY or subunits of twin arginine translocase (TAT) in their mitochondrial genomes. In bacteria, TAT complex transports substrates from cytosol to periplasm and is composed of three main subunits – TatA which is responsible for creating the transport channel and TatB, TatC which recognize the signal peptide of the substrate. A similar situation also exists in the chloroplast thylakoid. We investigated the evolution and function of mitochondrial TAT translocases in our current work. We show that mitochondrial TAT translocase was independently lost at least 21 times during the evolution of eukaryotes. By mapping the occurrence of mitochondrial TAT subunits in the rest of eukaryotes we define three types of TAT-derived machineries with different constituent components (TatABC, TatAC, and TatC-only). We show that mitochondria-encoded TatAC of the jakobid Andalucia godoyi represent the minimal functional pathway capable of substituting for the E. coli TatABC complex. However, selected TatC-only machineries were not capable of supporting the translocation of the substrate across the bacterial membrane.

FT7: miR-34c plays a key role during host-parasite infections in Theileria-infected macrophages and Plasmodium falciparum-infected red blood cells by targeting PRKAR2B

Haidar, Malak (KAUST, BESE DIVISION); *Ben-Rached, Fathia (Kaust); Langsley, Gordon (INSERM); Pain, Arnab (Kaust)*

MicroRNAs (miRNAs) are small non-coding RNA molecules that play critical roles in regulating eukaryotic gene expression including many parasitic infections, Here, we report the role of miR-34c in regulating PKA activity during Theileria and Plasmodium infection of leukocytes and erythrocytes respectiveLY. In this study, we provide experimental evidence for PRKAR2B (cAMP-dependent protein kinase type II-beta regulatory subunit) as a novel miR-34c target gene and plays a crucial role during host-parasite interactions during malaria and Theileria infections. Overexpression of miR-34c repressed PRKAR2B levels and consequently increased PKA activity in Theileria-transformed leukocytes promoting their virulent disseminating tumor phenotype. Similarly, P. falciparum-infection of red blood cells induces an increase in miR-34c-3p that ablates erythrocyte PKAR2B and PfPKAr expression leading to heightened activity of both erythrocyte and parasite cAMP-dependent PKA activity - the combination of which is important for invasion and subsequent intra-erythrocyte parasite development. This study sheds new light on our understanding of the roles of host miRNAs such as miR-34c that plays a role during infection by malaria and theileria parasites, and which may potentially be exploited as therapeutic targets.

FT8: Pentatricopeptide repeat factor functions as poly(A) binding protein in mitochondria of T. brucei

Mesitov, Mikhail (Department of Molecular and Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA)*; Yu, Tian (Department of Molecular and Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA); Suematsu, Takuma (Department of Molecular and Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA); Zhang, Liye (School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China); Huang, Lan (Department of Physiology and Biophysics, School of Medicine, University of California, Irvine, CA 92617, USA); Aphasizheva, Inna (Department of Molecular and Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA)*

In Trypanosoma brucei, most mitochondrial mRNAs undergo U-insertion/deletion editing, and 3'-end adenylation and uridylation. The internal sequence changes and terminal extensions are coordinated: pre-editing addition of short (A) tail protects edited transcript against 3'-5' degradation, while the post-editing A/U-tailing renders mRNA competent for ribosome recruitment. Nucleotidyl transferases contributing adenosines (KPAP1 poly(A) polymerase) and uridines (RET1 TUTase) have been identified, along with kinetoplast polyadenylation factors required for A-tailing (KPAF3) and A/U-tailing (KPAF1-2). Although participation of a poly(A) binding protein (PABP) in coupling of editing and 3' modification processes has been inferred in many studies, the identity of mitochondrial PABP remained elusive. Here, we report identification of an essential pentatricopeptide repeat-containing (PPR) Kinetoplast Polyadenylation Factor 4 (KPAF4), which fulfils in vitroproperties and biological functions of a poly(A) binding protein. We show that KPAF4 binding to A-tail stabilizes mRNA by impeding exonucleolytic activity of the mitochondrial processome. Conversely, KPAF4 binding inhibits RET1 TUTase-catalyzed uridylation of A-tailed transcripts and, therefore, premature A/U-tailing of pre-edited and partially-edited mRNAs. This quality check point ensures translational blockade of mRNAs lacking a complete protein coding sequence. Our findings also implicate the RNA editing substrate binding complex (RESC) in mediating a critical interaction between 5'-end bound pyrophosphohydrolase (PPsome) and 3'-end associated polyadenylation complex to enable mRNA circularization. This event appears to be critical for transcript stability during the editing process, while the post-editing A/U-tailing and translational activation likely require mRNA linearization. We will present a mechanistic model of mitochondrial mRNA surveillance by sequence-specific PPR proteins.

Poster Session A

Monday, September 10, 2018

7:00 pm – 9:00 pm

Poster Numbers 1 - 74

For Abstract see Session I, TT1
1: Identification of the Plasmodium falciparum var gene interactome with CRISPR-ChIP
Bryant, Jessica M. (Institut Pasteur)*; Baumgarten, Sebastian (Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris); SCHERF, Artur (Institut Pasteur)*

For Abstract see Session I, TT2
2: Using humanized malaria parasites to study protein interactions at the erythrocyte cytoskeleton
Warncke, Jan (Swiss Tropical and Public Health Institute, Basel, Switzerland); *Beilstein, Sabina; Wyss, Matthias; Gabel, Anke; Passecker, Armin; Perez, Lara; Butter, Falk; Beck, Hans-Peter*

For Abstract see Session I, TT3
3: In vivo and in vitro studies of the host-parasite interactions of Spironucleus salmonicida and the Atlantic salmon
Ástvaldsson, Ásgeir (Uppsala University) *Stairs, Courtney; Alfjorden, Anders; Hultenby, Kjell; Jansson, Eva; Svärd, Staffan*

For Abstract see Session I, TT4
4: Data mining and analysis using EuPathDB resources
Harb, Omar S; *Warrenfeltz, Susanne; Brunk, Brian; Kissinger, Jessica; Roos, David*

For Abstract see Session II, TT1
5: Consistent, comparative and evidence-based genome annotation and re-annotation for the closely-related species, Cryptosporidium parvum, C. hominis and C. tyzzeri
Baptista, Rodrigo P. (University of Georgia); *Li, Yiran; Sateriale, Adam; Ansell, Brendan; Jex, Aaron; Cotton, James A.; Sanders, Mandy; Brooks, Karen; Tracey, Alan; Berriman, Matthew; Striepen, Boris; Kissinger, Jessica C.*

For Abstract see Session II, TT2
6: Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to Giardia Intestinalis during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation
Ma'ayeh, Showgy Yasir (Uppsala University); *Stadelmann, Britta; Knörr, Livia; Sköld, Karin; Svärd, Staffan*

For Abstract see Session III, TT1
7: Targeting Cell Entry As Therapy for Leishmaniasis
Wetzel, Dawn M. (UT Southwestern Medical Center); *Ullah, Imran; Kernen, Rebecca M.; Booshehri, Laela M.; Rhodes, Emma L.; Mamula, Emily T.; Niederstrasser, Hanspeter; Posner, Bruce A.*

For Abstract see Session III, TT2
8: Cryptosporidium parvum exports proteins into the cytoplasm of the epithelial host cell
Dumaine, Jennifer E. (University of Pennslyvania); *Sateriale, Adam; Reddy, Amita; Striepen, Boris*

For Abstract see Session IV, TT1
9: Breaking the proventricular bottleneck: characterization of genes that enhance trypanosome migration to the tsetse salivary glands
Casas-Sanchez, Aitor (Liverpool School of Tropical Medicine); *Lopez-Escobar, Lara; Cansado-Utrilla, Cintia; Haines, Lee; Walrad, Pegine; Acosta-Serrano, Alvaro*

For Abstract see Session IV, TT2
10: Nutritional Supplementation Enhances Innate Immune Response Against Plasmodium Liver Stage Parasites
Meireles, Patricia (Instituto de Medicina Molecular Joao Lobo Antunes); *Bras, Daniela; Mendes, Antonio; Fontinha, Diana; Andrade, Carolina; Prudencio, Miguel*

11: Characterization of the perforin-like protein family in Babesia sp.

Paoletta, Martina S. (National Institute of Agricultural Technology)*; Jaramillo Ortiz, José (National Institute of Agricultural Technology); López Arias, Ludmila (National Institute of Agricultural Technology); Montenegro, Valeria (National Institute of Agricultural Technology); Suarez, Carlos (Washington State University - USDA); Farber, Marisa (National Institute of Agricultural Technology); Wilkowsky, Silvina (National Institute of Agricultural Technology)*

Bovine babesiosis is a tick-borne disease caused by protozoan parasites of the Babesia genus affecting livestock production worldwide. The study of proteins involved in parasite virulence is of special interest to understand the molecular basis of host-pathogen interaction and to develop better strategies to control the disease. The aim of this study was to characterize the Perforin-Like Protein (PLP) family of Babesia sp. These pore-forming proteins have not been characterized in the Babesia genus yet and its role could be critical for pathogenesis, as demonstrated in related apicomplexans. Here we identified by bioinformatics the plp genes in genomes of different Babesia species through detection of conserved motifs and domains. Six plp genes were found in B. bovis and B. ovata, while eight genes were found in B. bigemina and B. divergens. Phylogenetic analyses showed that pore-forming domains (MACPF) are highly conserved among Babesia species. Tertiary structure predictions revealed conformational conservation with other PLPs, suggesting similar functions with orthologs in the Apicomplexa phylum. Analysis of available transcriptomic data of B. bovis strains of contrasting phenotype showed that all plp genes are transcribed. The plp1 gene has the highest expression level and is the only one differentially expressed between attenuated and virulent strains. Western blot analysis of the recombinant MACPF domain of PLP1 shows that naturally infected bovine sera specifically recognize this domain confirming its immunodominance during natural infection. The hemolytic activity of the MACPF domain was confirmed through erythrocyte lysis assays, supporting its role in pathogenesis.Future work will be aimed to generate a plp1 knock-out B. bovis strain to determine the role of BboPLP1 in the parasite's virulence.

12: Pathogen screening from soft and hard ticks isolated from different species in Chihuahua, Mexico

Adame-Gallegos, Jaime R. (Universidad Autónoma de Chihuahua); *Beristain-Ruiz, Diana M. (Universidad Autónoma de Ciudad Juárez); Medrano-Bugarini, Raúl A. (Universidad Autónoma de Chihuahua); Lira-Amaya, José J. (CENID-PAVET / INIFAP); Rodríguez-Alarcón, Carlos A. (Universidad Autónoma de Ciudad Juárez); Figueroa-Millán, Julio V. (CENID-PAVET / INIFAP); Rivera-Chavira, Blanca E. (Universidad Autónoma de Chihuahua)*

After mosquitoes, ticks occupy the second place as vectors of zoonotic importance. Across Mexico, tick-borne diseases have become a matter of national concern in the last decade. The state of Chihuahua, southern neighbor of New Mexico and Texas, has had an increase of clinical cases of rickettsiosis since 2015. Most of the cases have been associated with Rickettsia rickettsii, etiological agent of Rocky Mountain spotted fever, as well as the presence of brown dog ticks Rhipicephalus sanguineus. In the last 3 years we have collected both hard (Ixodidae) and soft (Argasidae) ticks from different animal species living in endemic areas across the state. To this day, we have identified R. sanguineus, R. (Boophilus) microplus, Otobius megnini, Ornithodoros turicata, and Argas persicus from six different municipalities: Chihuahua, Nuevo Casas Grandes, Cuauhtémoc, Meoqui, Delicias, and Ciudad Juárez. So far, we have screened by PCR blood and ticks collected from horses, and identified Babesia equi, B. caballi, and Anaplasma phagocytophillum, that was also found in R. sanguineus ticks collected from dogs. We haven't had any positive results for R. rickettsii or Borrelia burgdorferi in the ticks screened. We aim to explore low-cost alternative tools to identify the specimens collected on-site, as well as faster identification of pathogens after DNA extraction. Our findings have shown that there is a mixture of tick species found in endemic areas, which may be involved in pathogen transmission. We plan to broaden our tick collection from municipalities with current tick infestation reports, and confirmed (or suspected) clinical cases.

13: Allosteric Site Inhibitor Disrupting Auto-activation of Cysteine Proteases of Plasmodium falciparum

Pant, Akansha (National Institute of Malaria Research); *kumar, Rajendra (Integrated Science Lab, Umeå University, Sweden); Wani, Naiem (Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Jammu); Verma, Sona (National Institute of Malaria Research, ICMR, New Delhi); Sharma, Ruby (School of Life Sciences, Jawaharlal Nehru University, New Delhi); Pande, Veena (Department of Biotechnology, Kumaun University, Nainital); Saxena, Ajay (School of Life Sciences, Jawaharlal Nehru University, New Delhi); Dixit, Rajnikant (National Institute of Malaria Research, ICMR, New Delhi); Rai, Rajkishore (Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Jammu); Pandey, Kailash (National Institute of Malaria Research, ICMR, New Delhi)*

Falcipains are hemoglobin degrading cysteine proteases of Plasmodium falciparum. They consist of pro- and mature domains that interact via 'hot-spot' interactions and maintain the structural integrity of enzyme in zymogen state. Upon sensing the acidic environment, these interactions dissociate and active enzyme is released. Several active site inhibitors have been designed to inhibit falcipain's activity, however compounds that target allosteric interactions remain uncharacterized. Therefore we synthesized first-in-class azapeptide based compounds. We tested their efficacy in P. falciparum 3D7 culture, inhibitors NA-01 and NA-03 showed effective EC50~1.2µM and 0.8µM, respectively. Compounds particularly inhibited the growth of trophozoites stage with swollen food vacuole. To find the site of binding, these inhibitors were examined for their auto-proteolysis, hemoglobin degradation and fluorogenic substrate activity. Activated falcipain incubated with these inhibitors were able to degrade hemoglobin suggested that the inhibitors did not bind to the catalytic site of the enzyme. These compounds showed high binding affinity in presence of prodomain with no change in the secondary structure. Our computational studies of molecular docking and molecular dynamics simulations suggested that inhibitors bind at pro- and mature domains interface, induces rigidity in the pro domain, and therefore prevent enzyme activation by resisting structural reorganization. Collectively, we provided a framework for targeting hotspot residues that could regulate falcipains in zymogen condition and halt its activation. This study may have futuristic approach to prevent pathogenic diseases and less sensitive to drug resistance.

14: Molecular dissection of Plasmodium falciparum importin a protein towards its assessment as a potential drug target

Dey, Vishakha (IIT Bombay); *Patankar, Swati (IIT Bombay)*

The first step towards a new antimalarial drug discovery is to find a potential target which can be impeded by a drug, thus preventing the disease. One such protein that merits assessment as a drug target for Plasmodium falciparum is importin α. Importin α is an adaptor protein in nucleocytoplasmic transport that not only recognises nuclear localisation sequence (NLS)-containing proteins, but also regulates the pathway through its property of auto-inhibition, mediated by its importin β binding (IBB) domain. Auto-inhibition is a universal feature in all importin αs studied so far from different organisms including all seven human importin αs, however with different extents of auto-inhibition. In contrast, Plasmodium falciparum codes for a single gene for importin α indicating that the nuclear transport machinery is solely dependent on a single importin α protein with no redundancy. Using in vitro binding assays and surface plasmon resonance, we show that the IBB domain of P. falciparum importin α lacks auto-inhibition. To study this unconventional facet of the protein, we generated a series of mutants, mimicking importin α that exhibit auto-inhibition and demonstrated that mutation of a single motif in the protein restores auto-inhibition. Our study, for the first time, deciphers the molecular mechanism along with quantitative measurement of an importin α that displays lack of autoinhibition. The deficiency of autoinhibition in P. falciparum importin α makes it distinct from human importin αs and would need novel strategies for NLS-cargo binding and release. Therefore, we propose that this protein could be an excellent choice as a potential anti-malarial drug target.

15: Molecular dissection of Plasmodium falciparum importin a protein towards its assessment as a potential drug target

Dey, Vishakha (IIT Bombay); *Patankar, Swati (IIT Bombay)*

The first step towards a new antimalarial drug discovery is to find a potential target which can be impeded by a drug, thus preventing the disease. One such protein that merits assessment as a drug target for Plasmodium falciparum is importin α. Importin α is an adaptor protein in nucleocytoplasmic transport that not only recognises nuclear localisation sequence (NLS)-containing proteins, but also regulates the pathway through its property of auto-inhibition, mediated by its importin β binding (IBB) domain. Auto-inhibition is a universal feature in all importin αs studied so far from different organisms including all seven human importin αs, however with different extents of auto-inhibition. In contrast, Plasmodium falciparum codes for a single gene for importin α indicating that the nuclear transport machinery is solely dependent on a single importin α protein with no redundancy. Using in vitro binding assays and surface plasmon resonance, we show that the IBB domain of P. falciparum importin α lacks auto-inhibition. To study this unconventional facet of the protein, we generated a series of mutants, mimicking importin α that exhibit auto-inhibition and demonstrated that mutation of a single motif in the protein restores auto-inhibition. Our study, for the first time, deciphers the molecular mechanism along with quantitative measurement of an importin α that displays lack of auto inhibition. The deficiency of auto inhibition in P. falciparum importin α makes it distinct from human importin αs and would need novel strategies for NLS-cargo binding and release. Therefore, we propose that this protein could be an excellent choice as a potential anti-malarial drug target.

16: Identification of cryptic stator subunits from an apicomplexan ATP synthase

Huet, Diego (Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA)*; Rajendran, Esther (Research School of Biology, Australian National University, Canberra, Australian Capital Territory); Van Dooren, Giel (Research School of Biology, Australian National University, Canberra, Australian Capital Territory); Lourido, Sebastian (Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA)*

Most eukaryotes possess a mitochondrial ATP synthase that uses the proton gradient generated by the electron transport chain to catalyze ATP synthesis from ADP and inorganic phosphate. The F1 catalytic portion of this macromolecular complex is linked to the Fo rotary portion in the inner mitochondrial membrane by the stator. Although proper function of the ATP synthase requires this link between F1 and Fo, sequence-based searches have failed to identify genes encoding the subunits of the stator in Apicomplexan parasites. Here, we identify 11 previously unknown subunits from the ATP synthase of T. gondii, which lack clear homologs outside of the apicomplexan phylum. Analysis of these sequences using hidden Markov modeling suggests that two of them—named ICAP2 and ICAP18—share distant homology with components of the ATP synthase stator. Biochemical and proteomic analyses show that both proteins interact with the know components of the ATP synthase. Conditional depletion of ICAP2 leads to aberrant mitochondrial morphology and disassembly of the complex, consistent with its key role as an essential component of the ATP synthase stator in T. gondii. Our findings highlight highly divergent features within the apicomplexan version of a central metabolic pathway, which will help identify therapeutic opportunities within these important human pathogens.

17: Unveiling crucial interactions between malarial cysteine proteases, falcipains and their natural substrate and macromolecular inhibitor

Pasupureddy, Rahul (National Institute of Malaria Research); *Verma, Sonia (National Institute of Malaria Research); Pant, Akansha (National Institute of Malaria Research); Sharma, Ruby (Jawaharlal Nehru University); Seshadri, Sriram (Nirma University); Saxena, Ajay (Jawaharlal Nehru University); Dixit, Rajnikant (National Institute of Malaria Research); Pandey, Kailash (National Institute of Malaria Research)*

Malarial cysteine proteases, falcipains, are responsible for degradation of hemoglobin, the principal source of nutrients for blood stage plasmodium parasites. Falcipains (falcipain-2 (FP2) and falcipain-3 (FP3)) interact with hemoglobin via a unique C-terminal motif, an exosite protruding away from active site cleft. Our study, through a combination of bioinformatic and mutagenesis analysis, has identified a single amino acid in both falcipains; Glu185 of FP2 and Asp194 of FP3, responsible for mediating interactions with hemoglobin. Residue swap experiments suggest that this position is functionally conserved among both falcipains. This approach could be advantageous for future drug development as the identified residue lies at an exosite protruding away from the active site, thus may likely be less susceptible to drug resistance. Further, we characterized the interactions between falcipains and their natural macromolecular inhibitor, falstatin, which plays crucial role during erythrocyte and hepatocyte invasion. Falstatin is unique as compared to its homologues, as only the BC loop is sufficient for the inhibition of falcipains. Our results suggest that falstatin interacts with FP2 in a multimeric form with ten units of falstatin interacting with ten units of FP2 in a 1:1 stoichiometry. Together, our study aims to comprehensively characterize the functioning of falcipains and interactions with their natural partners such as hemoglobin and falstatin, and explore novel chemotherapeutic targets.

18: Evidence of altered liver function and cytokine response profiles in pregnant women with malaria and chronic hepatitis B

Anabire, Nsoh Godwin (West African Centre for Cell Biology of Infectious Pathogens (WACCBIP); University of Ghana)*; Aryee, Paul (Department of Nutritional Sciences; University for Development Studies); Abdul-Karim, Abass (Zonal Public Health Laboratory, Tamale Teaching Hospital); Quaye, Osbourne (West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana); Awandare, Gordon (West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana); Helegbe, Gideon (Department of Biochemistry & Molecular Medicine, School of Medicine and Health Sciences, University for Development Studies)*

Background The overlap of malaria and chronic hepatitis B (CHB) is common in endemic regions, however, the impact of this co-infection on liver function and immune responses is unknown. This study sought to investigate these interactions in pregnant women reporting to antenatal clinics in Ghana.
Methods Levels of malaria parasitemia, hepatitis B viremia, liver biochemical parameters and inflammatory cytokines were assayed and compared across four categories of pregnant women: un-infected, mono-infected with Plasmodium falciparum (Malaria group), mono-infected with hepatitis B virus (CHB group) and co-infected with Plasmodium falciparum and hepatitis B virus (Malaria+CHB group). Results Relative to the CHB group, the Malaria+CHB group had lower viremia. However, levels of malaria parasitemia were similar in women in the Malaria and Malaria+CHB groups. Furthermore, levels of markers for liver injury/damage, including alanine aminotransferase, aspartate aminotransferase and total bilirubin were elevated in women in the Malaria+CHB group relative to those in the other groups. Similarly, pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6 were higher in women with Malaria+CHB compared to those in the other categories. In addition, pro-inflammatory cytokine levels had a significant positive correlation with viremia, and negative correlation with parasitemia. For anti-inflammatory cytokines including IL-10 and IL-4, the pattern was exactly the opposite of that for the pro-inflammatory cytokines.
Conclusion Plasmodium falciparum/CHB co-infection in pregnancy appeared to exacerbate the release of biomarkers for liver damage and inflammatory mediators while reducing immune-modulatory mediators. The exacerbated inflammatory response appears to help control malarial parasitemia in the co-infected women.

19: Identification and characterization of Toxoplasma genes that determine fitness in interferon gamma-stimulated human cells.

Krishnamurthy, Shruthi (University of California, Davis)*; Parades Santos, Tatiana (UCDavis); Sangare, Lamba Omar (UCDavis); Wang, Yifan (UCDavis); Sidik, Saima (Whitehead Institute for Biomedical Research); Lourido, Sebastian (Whitehead Institute for Biomedical Research); Saeij, Jeroen P. (UCDavis)*

The immune response against Toxoplasma involves the production of the cytokine interferon gamma (IFNγ), which can induce many different toxoplasmacidal mechanisms. IFNγ-mediated killing of Toxoplasma in mice is well understood but such is not the case in humans. The type I RH strain of Toxoplasma is relatively resistant to IFNγ-mediated clearance, but the exact mechanism remains unknown. To identify Toxoplasma genes that mediate resistance to IFNγ in human cells we used a whole genome loss-of-function screen using CRISPR/Cas9 technology. Briefly, we introduced a guide RNA library against all Toxoplasma protein coding genes into RHCas9 parasites to generate a pool of loss-of-function mutant parasites. We then used this pool of mutants to infect unstimulated or IFNγ-stimulated human cells. After several passages in each condition, we isolated DNA from the initial and final pools of parasites to determine which mutants were specifically depleted in IFNγ-stimulated human cells. We have identified multiple Toxoplasma genes that determine fitness in IFNγ-stimulated human cells of which we have validated one gene which is a putative apolipoprotein. We confirmed that the individual knockout parasites had a growth disadvantage compared to wild-type parasites only in the presence of IFNγ. We are currently determining the mechanism by which this gene product mediates resistance against IFNγ and are following up on other hits.

20: P-cyclins are master regulators of the Toxoplasma intermediate life cycle

Suvorova, Elena S. (University of South Florida); *Alvarez, Carmelo A. (University of South Florida); White, Michael W. (University of South Florida)*

Sporozoites in the environment and bradyzoites in contaminated food are the major sources of natural Toxoplasma gondii infections. Remarkably, we have found that these G1/G0 end-stage parasites initiate a similar developmental progression in human cells that occurs in nature; (1) a sporozoite or bradyzoite develops into a fast growing tachyzoite, and then within a week (2) slow dividing pre-bradyzoites emerge followed by (3) stochastic maturation of a dormant bradyzoite enclosed in a tissue cyst. Laboratory adapted strains reflect this pathway. RH strain is most similar to the fast growing tachyzoite that rapidly emerges from sporozoite infections, while many other strains (e.g. Pru, ME49, VEG) grow as pre-bradyzoites capable of spontaneous development into the mature tissue cyst. The molecular basis for developmental progression of intermediate life stages and the related differences in laboratory strains has never been determined. Utilizing a new auxin-induced conditional expression model (AID), we have discovered and experimentally confirmed that progressive action of three PHO80-family cyclins (P-cyclins), interacting with CDK-related kinase TgCrk2, likely regulates development of Toxoplasma intermediate life cycle and explains the different behavior of laboratory strains. The fast growing, developmentally incompetent RH strain is completely dependent on the first P-cyclin in the progression, TgCycP1, which is the only P-cyclin in sporozoites based on recent transcriptome data. By contrast, the slower growing and spontaneously developing ME49 pre-bradyzoite requires the second P-cyclin, TgCycP2 that is completely dispensable to the RH strain. The third P-cyclin in the series, TgCycP3, is essential to obtain a bradyzoite from the pre-bradyzoite stage. Through manipulation of P-cyclins levels, we demonstrated that we can reversibly steer Toxoplasma development. In pH-induced ME49 tachyzoites, downregulation of TgCycP3 blocked formation of the in vitro tissue cysts, but accelerated cyst maturation in the TgCycP2-deficient parasites.

21: High Cysteine Proteins play a major role during Giardia intestinalis interactions with host intestinal epithelial cells

Peirasmaki, Dimitra (Uppsala University)*; Ferella, Marcela; Höppner, Marc; Campos, Sara; Ankarklev, Johan; Stadelmann, Britta; Grabherr, Manfred; Svärd, Staffan (Uppsala University)*

Eukaryotic organisms are very complex where multiple kinds of cells and pathways taking place. For the survival and the well-being of the organism, it is essential that all different components and cells of it communicate sufficiently. Hormones and neurons are some of the most well-known participators in this type of communication. Nevertheless, excretory and secretory products (ESPs) secreted from many types of eukaryotic cells also serve as a way of communication where a direct cell to cell contact is not possible.Giardia intestinalis colonizes the small intestine of humans and animals causing diarrheal disease known as giardiasis. This single celled eukaryotic parasite does not internalize into host cells but it rather attaches to the villi in the small intestine disrupting the proper functioning of the epithelial barrier. In this project, we have used an in vitro model of the parasite interaction with the host intestinal epithelial cells (IECs) to study by RNA-seq genome wide changes in gene expression from Giardia, which might relate to persistence of infection and disease. The interaction between the parasite and the IECs was studied for 1.5h, 3h and 4.5h.Between hundred to 200 parasite genes presented altered mRNA levels. Among the highly up-regulated genes we found several members of the High Cysteine Protein (HCP) family, genes involved in cellular redox balance and genes from the lipid and nucleic acids metabolic pathways. In contrast, kinases, cell cycle and structural proteins were down-regulated, inferring a reduced cell proliferation. Hypothetical proteins were the major group detected as well in all three time points, indicating the vast number of important genes during infection which are still completely unknown. QPCR validated part of the RNA-seq and detected that the interaction media per se

induced some of the changes observed which were attenuated when IECs were present.In our RNA-seq, 20 to 24 members of the HCP family were highly up-regulated in all time points analyzed and therefore we decided to further study this uncharacterized family and its involvement in parasite-host interaction. Immunofluorescence microscopy of HA-tagged HCPs localized them to peripheral vesicles (PVs), plasma membrane and internal compartments.During interactions these proteins seemed to be secreted to the media.

22: Lipoate dependent enzymes of Plasmodium falciparum

Nair, Sethu C. (Johns Hopkins University); *Prigge, Sean (Johns Hopkins University)*

Blood stage malaria parasites cannot survive without scavenging the compound lipoate from the host. Lipoate acts as a cofactor for important enzymes and is an indicator of the redox balance inside the cell as well. In the current study, we are investigating the essentiality of enzymes that are dependent on scavenged lipoate. Three major enzyme complexes localized to the mitochondria uses lipoate as a cofactor. They are the mPDH, KDH and H-protein of the glycine cleavage system. Two mitochondrial enzymes catalyze the lipoylation of these proteins, LipL1 and LipL2. LipL1 mediates the lipoylation of H-protein, while mPDH and KDH are lipoylated by the sequential actions of LipL1 and LipL2. Using a CRISPR-based genetic knock out approach, we were able to delete KDH and mPDH (E2 and E1 subunits), but could not delete the H-protein. Consistent with this result, the enzyme responsible for H-protein lipoylation, LipL1, could not be deleted either, suggesting an essential role for lipoylated H-protein. Surprisingly, we were not able to delete LipL2 (responsible for KDH and mPDH lipoylation) unless high levels of acetate were added to the parasite growth medium. We then went on to generate a double knock out of mPDH and KDH and found that the double KO line was also dependent on acetate supplementation. This synthetic lethal phenotype suggests a redundancy of function between KDH and mPDH. Consistent with this conclusion, deletion of the E3 subunit shared by KDH and mPDH also resulted in an acetate-dependent parasite line. Taken together, these results suggest redundancy in the role of mPDH and KDH in generating acetyl-CoA for the parasite, and an essential role for lipoylated H-protein in some other process.

23: Toxoplasma proteins involved in nutrient acquisition across the parasitophorous vacuole membrane during acute and chronic stages

Paredes Santos, Tatiana (UCDavis); *Krishnamurthy, Shruthi (UCDavis); Sangare, Lamba (UCDavis); Wang, YiFan (UCDavis); Cardoso Barros, Patricio (UCDavis); Attias, Marcia (Federal University of Rio de Janeiro); Lourido, Sebastian (Whitehead Institute for Biomedical Research); Sidik, Saima (Whitehead Institute for Biomedical Research); Gold, Daniel (St. Edward's University); P Saeij, Jeroen (UCDavis)*

The parasitophorous vacuole membrane (PVM) of Toxoplasma gondii offers protection from the host immune system but also is a barrier for nutrient uptake. Our group showed that two dense granule proteins, GRA17 and GRA23, mediate the PVM permeability to small molecules such as nutrients. Because little is known about nutrient uptake across the cyst wall, our goal is to characterize the role of GRA17/23 in tissue cysts. We, therefore, made a type II ME49 GRA17knockout strain and showed that it has a "bubble vacuole" and reduced small molecule permeability through the PVM. The ME49Dgra17 parasites are able to form cysts in vitro but the number of in vitro cysts decreases 2-fold and viability of the parasites decreases 10-fold after 14 days of conversion. Because ME49Dgra17 parasites seem to be cleared before they reach the brain, to characterize the role of GRA17 in cyst persistence we are complementing the knockout strain with GRA17expressed from tachyzoite or bradyzoite-specific promoters. Besides GRA17/GRA23, other Toxoplasma gene products localized at the PVM are likely mediating transport of host nutrients into the PVM. We tried to identify these gene products using a genome-wide loss-of-function CRISPR/Cas9 screenin 'nutrient sensitized' parasite strains, such as the ?gra17 or ?gra23 strains. We identified genes that had large fitness defects in the ?gra17 or ?gra23 strains but not in wild-type. Many of these genes are involved in 1) vesicle transport, 2) transport of nutrients across membrane and 3) metabolism. We are currently confirming the top 25 hits of our screen. We expect to identify novel ToxoplasmaPVM-localized proteins that are involved in nutrient uptake across the PVM.

24: Developing a Detection Method for Trypanosoma congolense in Various Mouse Tissue Types

Pangburn, Sarah (CUNY Graduate Center, Hunter College)*; Verdi, Joey (CUNY Graduate Center, Hunter College); Raper, Jayne (CUNY Graduate Center, Hunter College)*

African trypanosomes are unicellular, eukaryotic parasites that live in the bloodstream of mammalian hosts and are transmitted via the tsetse fly. Animal infective trypanosomes cause animal African trypanosomiasis (AAT) in livestock in sub-Saharan Africa. Subspecies of the Trypanosoma brucei genre (T. b. rhodesiense and T. b. gambiense) can cause human African trypanosomiasis (HAT), also known as African sleeping sickness, in humans due to the crossing of the parasite over the blood brain barrier into the brain. Humans (and some other primates), are immune to all other animal infective trypanosome species due to the pore-forming protein Apolipoprotein LI (APOL1), which circulates in the blood as part of a HDL complex called trypanosome lytic factor (TLF). Transgenic mice expressing two copies of APOL1 are immune to animal infective trypanosomes, whereas heterozygous transgenic mice develop T. congolense infections 25% of the time due to a sublethal dose of APOL1. These resistant parasites could be evolving in tissue types with low HDL concentrations, such as adipose tissue. Recent work has shown that T. brucei parasites exist in adipose as adipose tissue forms, however, characterization of other animal infective parasites in various tissue types has not been previously identified. T. congolense resistant parasites are not detectable in the mice until parasitemia rises due to the limit of detection when parasitemia is counted by eye, therefore, a more sensitive approach is needed to detect these parasites in various tissues types. In this study, we have used qPCR and T. congolense specific primers to detect T. congolense parasites in multiple mouse tissues.

25: Identification of metabolic pathways that regulate sexual differentiation in P. falciparum.

Sollelis, Lauriane (Wellcome Centre for Molecular Parasitology)*; Straub, Timothy J (The Broad Institute of MIT and Harvard); De Niz, Mariana; Laffitte, Marie-Claude (Wellcome Centre for Molecular Parasitology); Brancucci, Nicolas M B; Barrett, Michael; Neafsey, Daniel E; Marti, Matthias (Wellcome Centre for Molecular Parasitology)*

Malaria remains a burden in endemic countries, where the disease continues to cause over 400, 000 deaths each year. During asexual replication of the parasite in red blood cells a subset of parasites commit to a sexual differentiation pathway and become gametocytes, the only form transmissible to mosquitoes. Sexual commitment is induced upon activation of a transcriptional master switch, ag2-g, in the parasite (Sinha et al., 2014). We have recently demonstrated that ag2-g activation is induced by limiting the host phospholipid lysophosphatidylcholine (LysoPC)(Brancucci et al., 2017). Using transcriptional and metabolomic analyses we were able to define the signature of sexual commitment upon LysoPC restriction. These data demonstrated compensatory upregulation of ethanolamine kinase (ek) and phosphoethanolamine-methyl-transferase (pmt) involved in de novo phospholipid biosynthesis through the Kennedy pathway (Dechamps et al. 2010). Notably, pmt is absent in the rodent malaria parasite and this lineage is also insensitive to LysoPC restriction. Therefore, we hypothesized that pmt is required for the sexual commitment pathway. In order to understand the role of pmt in the context of sexual differentiation we have generated a pmt knock out by CRISPR/Cas9. Surprisingly the deletion of pmt did not alter sexual commitment rates despite severe growth defect in absence of LysoPC. However, further analysis revealed a defect during gametocyte development, in accordance with previously observed transmission defects (Bobenchik et al., 2013). We are currently analysing the pmt ko parasites by RNAseq, metabolomics and quantitative imaging to further define its role during the parasite blood stage and transmission to mosquitoes.

26: Structural studies of the interaction between FPC3 and FPC4, two essential components of the flagellar pocket collar in Trypanosoma brucei

***Majneri, Paul**; Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria); Pivovarova, Yulia; Landrein, Nicolas; Lesigang, Johannes; Robinson, Derrick; Bonhivers, Mélanie*

Sub-species of the unicellular parasite Trypanosoma brucei are the causative agent of Human African Trypanosomiasis (sleeping sickness) that threatens millions of people in all sub-Saharan countries. T. brucei has a unique flagellar pocket at the base of its flagellum, which is the sole site of all endo- and exocytic activities in the cell and has thus been proposed to be a therapeutic target. At the neck of the flagellar pocket is a belt-like cytoskeletal structure called the flagellar pocket collar (FPC). We have previously characterized the structure and assembly of a multi-domain cytoskeletal and essential protein named BILBO1, which serves as an anchor for the docking of other FPC components to maintain the structure and function of the FPC in T. brucei.
Several FPC proteins have been newly identified; one of them is called FPC3, which shares ~30% sequence identities with BILBO1 within their N-terminal domains (NTD). Here we show that FPC3 is a FPC protein that co-localizes with BILBO1, and both yeast two-hybrid and biochemical studies demonstrate that FPC3-NTD interacts with the C-terminal domain (CTD) of FPC4, a previously characterized BILBO1 partner protein. We have managed to co-crystallize FPC3-NTD together with FPC4-CTD. The structure was determined by molecular replacement to 1.6-Å resolution, which represents the first ever high-resolution structure between different FPC proteins. The structure shows that FPC3-NTD adopts a similar fold to that of BILBO1-NTD reported previously, and an extended segment of FPC4 binds tightly to the conserved hydrophobic pocket on the globular FPC3-NTD. Further mutagenesis analyses in combination with affinity determination using isothermal titration calorimetry (ITC) revealed the residues crucial for their interaction. Overall, our structural work provides a clear view of the interaction between two FPC proteins, which may be used to guide therapeutic design of inhibitors of either modified polypeptides or small molecules to disturb the binary interaction in vivo.

27: Lathosterol oxidase is important for acid resistance and lipophosphoglycan synthesis in Leishmania major

Ning, Yu (Texas Tech University); *Hsu, Fong-Fu (Washington University); Zhang, Kai (Texas Tech University)*

Sterols are essential membrane components and precursors for the synthesis of bioactive molecules in eukaryotes. Several enzymes in the sterol biosynthetic pathways are drug targets to treat diseases caused by pathogenic fungi and trypanosomatids. In Leishmania major, sterol C14-demethylase and sterol methyl transferase play crucial roles in the maintenance of plasma membrane stability, heat resistance, and the production of reactive oxygen species. To elucidate how sterols control stress response in Leishmania, it is necessary to examine the roles of other enzymes in sterol synthesis. In this study, we characterized the lathosterol oxidase (LSO) in L. major, which catalyzes the generation of C5-C6 double bond on sterol intermediates, a ubiquitous step in the sterol synthesis of all eukaryotes. Deletion of LSO leads to a complete loss of the C5-C6 double bond on sterols. Despite this change in sterol synthesis, LSO-null mutants are fully viable and replicative in culture, but show poor survival in stationary phase and reduced ability to form metacyclics. In addition, LSO is required for cell growth under acidic pH and the synthesis of lipophosphoglycan (LPG), a GPI-anchored virulence factor. LSO-null mutants also display mitochondrial abnormalities including increased mitochondria membrane potential, higher superoxide production and lower oxygen consumption. Future studies will determine the mechanism by which LSO affects acid resistance and LPG synthesis in Leishmania.

28: High throughput screening to identify selective proteasome inhibitors as new antimalarials with a novel mode of action

Mata-Cantero, Lydia (GlaxoSmithKline)*; Cortés, Álvaro (GlaxoSmithKline); García, Mercedes (GlaxoSmithKline); Xie, Stanley (University of Merlbourne); Gillett, David (University of Melbourne); Gamo, Javier (GlaxoSmithKline); Tilley, Leann (University of Melbourne); Gómez, María G. (GlaxoSmithKline)*

The most severe form of malaria is caused by the protozoan parasite Plasmodium falciparum. Current first-line treatment is based on artemisinin combination therapies (ACTs). However, the malaria parasite has developed resistance against all widely used antimalarials, and ACTs are not an exception. Increasing drug resistance has led to an urgent need for developing new therapies acting though new parasite targets that are effective not only on acute infection, but also on different stages of the parasite to block transmission. Proteasome fulfill these criteria, being one of the best validated targets in malaria. Human proteasome inhibitors used for cancer treatment are active on all parasite stages, liver, blood (sexual and asexual) and mosquito. Moreover, they show a synergistic behavior in sensitive or resistant parasites when combined with artemisinin. Although P. falciparum proteasome exhibits a high degree of homology with its human counterpart some divergences have been found, including an unusually open β2 active site. These divergences are being exploited to develop inhibitors with selectivity for the parasite proteasome, thus limiting the potential side effects derived from human proteasome inhibition. Here, we present a highthroughput screening campaign with around 500K compounds from GSK collection using purified P. falciparum proteasome. Chymotrypsin-like activity has been used as primary assay to filter the compounds, followed by assays to test trypsin and caspase-like activities, as well as selectivity evaluation with human proteasome. Results from the primary screening, validation in secondary and selectivity assays, as well as the progression cascade to validate biochemically and biologically the hits will be presented.

29: A Toxoplasma Oxygen Sensing Protein Mediates Cellular Responses to Hyperoxic Stress and is Required for Tissue Specific Dissemination

***Blader, Ira**; Florimond, Celia; Cordonnier, Charlotte; Taujale, Rahil; va der Wel, Hanke; Kannan, Natarajan; West, Chris*

Toxoplasma is a food-borne pathogen that causes disease in fetuses and immunocompromised individuals. As Toxoplasmadisseminates through its host, it must respond to the diverse environments it encounters and does so by altering its gene expression, metabolism, and other processes. Oxygen is one variable environmental factor and properly adapting to changes in oxygen availability is critical to prevent the accumulation of reactive oxygen species and other cytotoxic factors. Thus, oxygen-sensing proteins are important and amongst these the 2-oxoglutarate-dependent prolyl hydroxylases are highly conserved throughout evolution. Toxoplasmaexpresses two such enzymes, TgPHYb and TgPHYa, which regulates the SCF-ubiquitin ligase complex. To characterize TgPHYb, we created a Toxoplasmastrain that conditionally expresses TgPHYb and find that TgPHYb is required for optimal parasite growth. TgPHYb is dispensable for parasite invasion and replication but is required for survival of extracellular parasites. In contrast to most other oxygen-sensing proteins that mediate cellular responses to low O2, TgPHYb is specifically required for parasite growth at high, but not low, O2tensions. In vivo, reduced TgPHYb expression leads to reduced parasite burdens specifically in oxygen-rich tissues. Extracellularly stressed TgPhyB-depleted parasites display decreased rates of protein synthesis, which prevents replenishment of invasion proteins spontaneously released by extracellular parasites. Here we report that TgPHYb regulates translation elongation. Taken together, these data identify TgPHYb as a sensor of high O2, in contrast to TgPHYa which supports the parasite at lowO2.

30: Identification of regulatory factors that determine Toxoplasma bradyzoite to merozoites conversion

***Arranz-Solís, David (UC Davis)**; Mukhopadhyay, Debanjan (Uc Davis); Saeij, Jeroen (UC Davis)*

Cats are the definitive host for Toxoplasma gondii. When this parasite invades the cat intestinal epithelium, it differentiates into merozoites that will convert into male/female gametes. Upon fusion, extremely resistant and infectious oocysts will be formed and shed within cat feces. Despite the crucial epidemiological role of cat intestinal stages, these are the least characterized because they are not cultivatable in vitro and difficult to access in vivo. Therefore, our goal is to investigate the molecular mechanisms governing the initiation of Toxoplasma's development into its feline intestinal stages. For this we are using the M4 type II strain, from which we have generated Δhpt, Δuprt, and Cas9 expressing strains that still form oocysts. To be able to visualize and select merozoites, a merozoite reporter parasite line expressing the pyrimethamine-resistance cassette and the red-fluorescence marker tdTomato was generated. Moreover, we engineered transgenic parasites in which putative regulatory factors involved in bradyzoite-merozoite conversion can be artificially expressed in vitro by means of a tetracycline-inducible expression system. We first focused on genes encoding for AP2-like transcription factors, such as AP2IV-3, the ortholog of Plasmodium PfAP2-G, a sexual-stage master regulator. We confirmed the expression of AP2IV-3 by immunofluorescence only after addition of tetracycline. We will further investigate if the upregulation of this or other factors, alone or in combination, can induce the conversion to merozoites by detecting expression of tdTomato and/or selection with pyrimethamine and by transcriptional profiling. If we succeed, this will be the first step towards completing the entire intestinal development in vitro, allowing the assessment of the molecular mechanisms governing the initiation of Toxoplasma's feline intestinal stages.

31: Unconventional ribosomes with highly fragmented rRNAs in malaria parasite mitochondria

Ke, Hangjun (Drexel University College of Medicine); *Dass, Swati (Drexel University College of Medicine); Mulaka, Maruthi (Drexel University College of Medicine); Gutierrez-Vargas, Cristina (Columbia University); Mather, Michael (Drexel University College of Medicine); Dvorin, Jeffrey (Boston Children's Hospital); Frank, Joachim (Columbia University); Vaidya, Akhil (Drexel University College of Medicine)*

Apicomplexan parasites are unicellular protozoans possessing a single mitochondrion per parasite. Compared to animals, fungi, plants and some other Alveolate groups, apicomplexans have a reduced mitochondrion in terms of size and function. In most apicomplexans, the mitochondrial genome (mtDNA) is a linear molecule of 6-11 kb encoding only three proteins, cyt b, COXI, COXIII. It also encodes many highly fragmented small rRNA genes, but no tRNA genes. The 6 kb malarial mtDNA is known as the smallest organellar genome among all eukaryotes. To translate the mtDNA-encoded genes, Plasmodium utilizes a special ribosome in the mitochondrion, the mitoribosome. Mitoribosomes, in general, are distinct from cytoplasmic and prokaryotic ribosomes. They are highly porous in structure and have reversed the ratio of ribosomal proteins over rRNAs from 1:2 in other ribosomes to 2:1. Compared to yeast or human mitoribosomes, however, the mitoribosomes in malaria parasites are even more unique, since the RNA component is composed of many small fragmented rRNAs, rather than two large rRNA molecules. As rRNAs are the enzymatic entity in ribosomes, it remains entirely unknown how small fragmented rRNAs form a functional ribozyme. Moreover, relatively few mitoribosomal protein genes have been annotated in the malarial genomic databases. Herein, we aim to dissect the protein and RNA compositions and resolve the structures of mitoribosomes in malaria parasites. To investigate these aims, we will employ genetic, biochemical and structural tools. The compositional and structurally unique features of the malarial mitoribosomes could be exploited for developing antimalarial drugs in future.

32: Sterol synthesis has major impact on the mitochondrial physiology and stress response in Leishmania major.

Mukherjee, Sumit (Texas Tech University); *Xu, Wei (Washington University at St. Louis); Zhang, Kai (Texas Tech University)*

Like pathogenic fungi, Leishmania parasites synthesize ergosterol and other C24-methylated sterols. The C14-α demethylase (C14DM) is a key enzyme in sterol synthesis and an important target for azole drugs. Deletion of C14DM significantly alters the sterol composition in L. major leading to severe defects when parasites are challenged with mild heat, osmolality stress or starvation. To explore how sterol synthesis affects stress response in Leishmania, we examined the plasma membrane and mitochondria in the C14DM-null mutants. Results showed that the mitochondria in C14DM mutants have significantly higher inner membrane potential (Ψm) than the wild type (WT) parasites. Such high Ψm is responsible for accumulation of superoxide in the mitochondria of the mutants, which may cause cellular damage. The accumulation of superoxide, along with increased membrane fluidity, appears to be responsible for the heat sensitivity in the C14DM mutants. On the other hand, the starvation defect seems to be mainly due to perturbation of autophagy as the mutants failed to form autophagosomes as efficiently as the WT parasites. Thus alteration of sterol synthesis affects multiple cellular processes in Leishmania. Further studies will elucidate how sterol synthesis controls the content, function and dynamic of mitochondria in Leishmania parasites.

33: Development of CRISPR/Cas9-mediated disruption systems in Giardia lamblia

Sun, Chin-Hung (National Taiwan University); *Lin, Zi-Qi (College of Medicine, National Taiwan University,); Gan, Soo-Wah (College of Medicine, National Taiwan University); Tung, Szu-Yu (College of Medicine, National Taiwan University); Ho, Chun-Che (College of Medicine, National Taiwan University); Su, Li-Hsin (College of Medicine, National Taiwan University)*

Giardia lamblia, an enteric protozoan pathogen, becomes dormant by forming infectious cysts. During encystation, cyst wall proteins (CWPs) are highly expressed to form cyst wall. The tetraploid genome in the two nuclei makes it complicated to do gene knockout for molecular characterization. Development of a clustered regulatory interspersed short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system is in urgent need for studies of this organism. We developed a CRISPR/Cas9 system with two plasmids expressing Cas9 and guide RNA separately using puromycin selection for targeting the myeloid leukemia factor (mlf) gene in Giardia. We generated only knockdown but not knockout mutants with the gene disruption efficiency of about 32%. In addition, knockdown of the mlf gene significantly decreased cwp gene expression and cyst formation, suggesting a positive role of MLF in encystation. To further improve gene disruption efficiency on mlf, we added an inhibitor for non-homologous end joining, Scr7, to promote homologous recombination (HR), and found that addition of Scr7 increased the gene disruption efficiency to about 53%. We also established a CRISPR/Cas9 system with neomycin selection for use in complementation studies by transfecting MLF expression vector with puromycin selection. In addition, combining the Cas9 and guide RNA expression cassettes, and HR template into one plasmid increased the gene disruption efficiency to about 70%. Our results provide a useful CRISPR/Cas9 system as an improved tool for functional analysis in Giardia studies.

34: A myeloid leukemia factor-like protein involved in encystation-induced protein metabolism in Giardia lamblia

Sun, Chin-Hung (National Taiwan University); *Wu, Jui-Hsuan (College of Medicine, National Taiwan University); Lin, Zi-Qi, (College of Medicine, National Taiwan University); Su, Li-Hsin (College of Medicine, National Taiwan University); Gan, Soo-Wah (College of Medicine, National Taiwan University); Liao, Jo-Yu (College of Medicine, National Taiwan University); Tung, Szu-Yu (College of Medicine, National Taiwan University)*

Giardia lamblia must encyst into water-resistant cysts which can be a good model for dormancy. However, little is known about its regulation mechanism. Myeloid leukemia factor (MLF) proteins are important regulators of cell differentiation, apoptosis, and tumorigenesis in mammals. We found that Giardia possesses a conserved MLF homolog which was up-regulated during encystation and localized to unknown cytosolic vesicles named MLF vesicles (MVs). The MVs are not mitosomes or encystation-specific vesicles as double staining with ISCU or CWP1 revealed no overlap between these vesicles. An aberrant protein, CDK2m3, colocalized with the MVs and formed complexes with MLF. Addition of MG132, a proteasome inhibitor, increased the number of vesicles and level of CDK2 mutant, suggesting that CDK2m3 is degraded by the protein degradation or autophagy pathway. We further found a colocalization of the MVs with a proteasome component, RPN11, and with two autophagy-related factors, a FYVE protein and ATG8. Co-immunoprecipitation analysis revealed a complex formation of MLF with RPN11, FYVE, or ATG8 protein. Interestingly, all these proteins were up-regulated during encystation and induced Giardia encystation, suggesting that they are in the same pathway. Addition of chloroquine, an inhibitor for proteasome and autophagy, increased the levels of MLF, FYVE, and ATG8 proteins and the number of their vesicles, suggesting that they function in degradative or autophagy pathway.

Starvation also increased the levels of MLF, FYVE, and ATG8 proteins. Proteasome components, MLF, and FYVE were detected in exosomes release from culture, supporting the idea that exosomes and degradative/autophagy may be coordinated. Our results provide evidence

that MLF may be involved an encystation-induced protein metabolism in Giardia for differentiation into cysts.

35: PfSR1 is essential for DNA damage repair and resistance to artemisinin in Plasmodium falciparum

Dzikowski, Ron (Hebrew University)*; Singh, Brajesh (Hebrew university); Goyal, Manish (Hebrew University); Siman-Tov, Karina (Hebrew University); Kaufman, Yotam (Hebrew University); Eshar, Shiri (Hebrew University)*

Plasmodium falciparum, the parasite responsible for the deadliest form of human malaria, maintains this complex life cycle with a relatively small number of genes. One way by which eukaryotic organisms may expand their protein repertoire out of relatively small number of genes is by Alternative Splicing (AS) of pre-mRNA molecules. Nevertheless, the regulation of the AS machinery in Plasmodium spp. is still elusive. We have previously showed that PfSR1 is a putative SR protein that functions as an alternative splicing factor in vivo, shuttles between the nucleus and cytoplasm, and preferentially binds specific RNAs motifs. To further investigate the regulatory role of PfSR1 in gene expression in P. falciparum we perform a stage dependent pull down assay to unveil its interacting proteins. We found that PfSR1 interacts with proteins which are linked to various processes of RNA metabolism in a stage dependent manner. These include: chromatin re-modeling and transcription, splicing and translation. Intriguingly, some of PfSR1 interacting proteins are orthologues to proteins implicated in DNA damage repair. We found that following DNA damage, PfSR1 co-localized and interacts with ?H2A indicating its association with damaged loci. In addition, PfSR1 expression was found to be essential for parasite recovery from induced DNA damage as well as from exposure to artemisinin. Altogether these findings point towards a novel role of PfSR1 in protecting P. falciparum from DNA damage and artemisinin exposure.

36: Structural characterisation of the merozoite surface protein-1 using recombinant proteins from different Plasmodium strains

Marzluf, Tanja (Centre of Infectious Diseases, Parasitology Department)*; Bujard, Hermann (ZMBH, Heidelberg, Germany)*

P. vivax is the most widespread species and is responsible for more than 50% of malaria cases in non-African regions. However, vaccine development for P. vivax has received little attention. One reason is the absence of a continuous cell culture system, a major obstacle for biomedical research on this parasite. A possible strategy is to examine P. vivax orthologs of P. falciparum vaccine candidate antigens for their potential use as a vaccine. The Merozoite Surface Protein 1 (MSP-1) from P. falciparum is considered a promising vaccine candidate because it plays an essential role during erythrocyte invasion and has been associated with protection against malaria in various studies. Remarkably, the overall structure of MSP-1 of P. vivax displays a high degree of similarity to MSP-1 of P. falciparum. This similarity in terms of sequence and, most likely, function makes this protein a candidate for the development of a P. vivax vaccine as well. However, little is known about the structural features of MSP-1. Gaining more knowledge about its three dimensional structure and interaction sites could contribute significantly to improved immunisation strategies. Targeting common structural features could result in protection against multiple Plasmodium species. To identify such key elements, protein production protocols were developed to obtain recombinant proteins based on P. falciparum, P. vivax and P. knowlesi MSP-1. In contrast to most other studies, the recombinant MSP-1 proteins represent the native protein in full length. Biochemical and biophysical assays, such as CD spectroscopy, proteolytic processing and crosslinking were used to detect shared structural features and interaction sites.

37: The Role of BEI Resources as a Centralized Repository for Parasitology Research

Molestina, Robert (ATCC/BEI Resources)

Protozoan parasites have evolved a series of strategies to evade the immune system and establish chronic infections in the host. Elucidating the underlying mechanisms used by highly adapted parasites to block or subvert host processes offers new targets for therapeutic or vaccine development. In this setting, the accessibility of reference strains and specialized reagents is critical to the generation of studies aimed at deciphering the intricate aspects of the host-parasite relationship. Protozoan strains deposited in centralized repositories are in fact considered 'biological standards' as they are critical components of comparative studies. BEI Resources houses a large and diverse repository of parasitic protozoa and related reagents for the worldwide scientific community. The primary role of BEI Resources since 2003 has been the acquisition, authentication, preservation, and distribution of reference parasite strains. Characterization of deposited cultures includes a variety of tests, including viability, purity, and phenotypic and genotypic analysis. To assure that identity and performance of BEI Resources reagents meet quality standards, the characterization methods used are reliable, applicable to the widest variety of taxa, and reproducible. Establishment of seed and distribution stocks for every strain is a common practice to ensure that cultures distributed to researchers are minimally passaged from the original material provided by the depositor. Over the last decade, catalog holdings have expanded to include a variety of biomaterials such as transgenic parasites expressing various reporter genes, knockout strains, polyclonal and monoclonal antisera, genomic DNAs, and expression vectors. An overview of the biological resources available to the researcher, the benefits of depositing and registering with BEI Resources, current projects in the program, and future perspectives will be presented.

38: The Proteome of Malaria Parasite Merosomes and Liver Stage Merozoites

Shears, Melanie J. (Johns Hopkins University)*; Nirujogi, Raja; Swearingen, Kristian (Institute for Systems Biology); Renuse, Santosh (Johns Hopkins University); Mishra, Satish; Mortiz, Robert (Institute for Systems Biology); Pandey, Akhilesh (Johns Hopkins University); Sinnis, Photini (Johns Hopkins University)*

Malaria parasites must replicate in the liver before initiating the symptomatic blood stage of infection. Liver stage replication produces liver stage merozoites, which are released into the bloodstream in packets called “merosomes” that are surrounded by host-derived membrane. The liver stage is recognized as an important target for anti-malarial drugs and vaccines, and there is a strong need to explore the basic biology of this stage. However, little is known about liver stage merozoite biology, or the host-parasite interactions required for merosome formation and release. Here we use the Plasmodium berghei rodent malaria model to generate the first ever proteome of merosomes and the liver stage merozoites within. We confidently identify 1188 parasite proteins, providing a comprehensive picture of the protein repertoire of this stage. Comparison to published liver and blood stage proteomes identifies broad commonalities with these stages, and reveals liver and blood stage merozoites share the majority of characterized merozoite proteins. Importantly however, we find evidence that liver and blood stage merozoites differentially express at least one merozoite surface protein, identifying a previously unknown distinction between these two merozoite types. Finally, the discovery of several proteins with processed protein export motifs gives new insight into the mechanism of protein export in the liver stage. This work therefore considerably expands our understanding of liver stage merozoite biology and host-parasite interactions during liver stage development.

39: Thymidylate synthase validation as antimalarial target using CRISPR-Cas9

Moliner-Cubel, Sonia (GlaxoSmithKline)*; Palomo-Diaz, Sara (GlaxoSmithKline); Franco-Hidalgo, Virginia (GlaxoSmithKline); Winzeler, Elizabeth (University of California San Diego); Gomez-Lorenzo, Maria (GlaxoSmithKline); Gamo, Javier (GlaxoSmithKline)*

Resistance to all known antimalarial drugs has been reported, highlighting the urgent need to discover new chemical entities with novel modes of action. Target based screenings are an excellent way to identify novel chemical diversity to enable development of new antimalarial drugs. However, in malaria this approach is restricted due to the limited number of genetically or pharmacologically validated drug targets. The dihydrofolate reductase (DHFR) domain of the dihydrofolate reductase-thymidylate synthase (DHFR-TS) bifunctional polypeptide is the target of pyrimethamine and has been fully validated as antimalarial target. Conversely, the essentiality and drugability of the thymidylate synthase (TS) domain remains to be validated. Unlike the human host cells, the malarial parasite cannot salvage pyrimidines and relies on their de novo biosynthesis. The TS domain catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP).In vitro resistance selection studies using a phenotypic hit (MMV027634), with unknown mode of action, revealed mutations in the TS domain of the DHFR-TS gene. To confirm if the mutations identified are the cause of decreased sensitivity to the compound, we used CRISPR-Cas9 technology. We generated parasite lines expressing mutant proteins containig the amino acid changes I403L, G378E or H551N identified in the DHFR-TS gene during selection studies. Parasite resistance was verified using IC50 assays against the selection compound. These three mutations also confer resistance to a structural analog of MMV027634 found in GSK compound collection. Noteworthy, only mutations G378E and H551N confer resistance to 5-fluoroorotate (5-FoA), a metabolic precursor of 5-fluoro-2'-deoxyuridylate that inhibits the P. falciparum TS activity. Altogether, our results further support validation of the TS domain as an antimalarial target.“The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.”

40: Defining a transcriptional repressor complex controlling tissue cyst development in Toxoplasma gondii

Srivastava, Sandeep (Indiana-Purdue University); *Huang, Sherri (Indiana-Purdue University); Holmes, Michael J. (Indiana-Purdue University); Radke, Joshua B. (Department of Global Health, University of South Florida, Tampa, Florida, USA); Hong, David (Department of Global Health, University of South Florida, Tampa,Florida); Liu, Ting-Kai (Indiana-Purdue University); White, Michael W. (Department of Global Health, University of South Florida, Tampa, Florida, USA); Sullivan Jr, William J. (Indiana-Purdue University)*

Toxoplasma gondii is an intracellular protozoan parasite of animals and humans that causes severe disease in immunocompromised individuals and long term chronic infections in healthy people due to its ability to form latent tissue cysts. T. gondii alters ~5% of its transcriptome during tissue cyst formation through mechanisms that are not fully understood. We previously identified an ApiAP2 transcription factor called AP2IX-4 (TGME49_288950) that is expressed only in dividing parasites. Genetic knockout of AP2IX-4 had no discernible effect on the proliferating form of the parasite (tachyzoite stage) but reduced the frequency of tissue cyst formation (bradyzoite stage) in vitro and in a mouse model of infection. Transcriptional profiling studies show that ablation of AP2IX-4 results in the upregulation of a subset of bradyzoite genes, which indicates AP2IX-4 is transcriptional repressor. Consistent with this transcriptional function, co-immunoprecipitation of epitope-tagged AP2IX-4 (3xHA) interacts with CRC230 (a subunit of the TgCRC co-repressor complex) and histone deacetylase 3 (HDAC3), along with two additional ApiAP2 proteins (AP2XII-2 and AP2VIIa-3) and a hypothetical protein (TGTT1_214140). Components of the AP2IX-4 complex are being epitope tagged with 3xMyc in order to confirm these interactions in tachyzoites by reciprocal co-immunoprecipitation (AP2IX-4 is HA tagged). Ongoing studies are examining whether the composition of the AP2IX-4 complex changes when tachyzoites are induced to switch to bradyzoites.

41: TURNOVER OF VSG IN TRYPANOSOMA BRUCEI

***Garrison, Paige**; Umaer, Khan; Bush, Peter; Bangs, Jay*

Trypanosoma brucei utilizes the critical virulence factor, Variant Surface Glycoprotein (VSG), to evade the immune system and maintain persistent infection in the mammalian host. VSG is attached to the plasma membrane by glycosylphosphatidylinositol (GPI) anchors. VSG turnover is thought to be due to GPI cleavage by GPI-specific Phospholipase C (GPI-PLC), releasing VSG from the cell in soluble form (Bulow et al., 1989, Mol Biochem Parasitol, pp85-92). However, recent work (Szempruch et al., 2016, Cell, pp246–257) suggests that VSG is also released on nanotubes, which bud from the surface to form free extracellular vesicles (EVs). We evaluate the extent to which GPI-PLC cleavage and nanotube budding contribute to overall VSG turnover in WT and GPI-PLC null cell lines. Pulse-chase experiments indicate turnover at t1/2 ~30 hr in WT cells, with quantitative recovery in the media, in good agreement with the earlier studies. Interestingly, VSG turnover remains unchanged in GPI-PLC null cells, but recovery in the media is significantly reduced. In each case the majority of the released VSG is floatable on density gradients indicating inclusion in membranous EVs. Quantitation by SEM indicates no difference between WT and GPI-PLC nulls in number, size or location of budding nanotubes. The discrepancy in recovery is currently under investigation. In agreement with previous data (Subramanya et al., 2009, Biochem J, pp685-694), GPI-PLC null cells also exhibit ~60% reduction in transferrin uptake, without effecting lysosomal delivery or transferrin receptor turnover rate. Finally, VSG turnover in unchanged when VSG is subject to RNAi silencing, but SEM suggests that the cell limits its surface area to maintain constant VSG density/packing. Our results indicate that shedding of VSG containing membrane, not GPI-hydrolysis, is the main mode of VSG turnover.

42: Proximity-dependent biotin labeling reveals the spatial organization of the Plasmodium DOZI/CITH/ALBA complex

Rios, Kelly (Pennsylvania State University); *Lindner, Scott (Pennsylvania State University)*

Plasmodium transmission between mammalian hosts and the mosquito vector is integral to the parasite's lifecycle and the spread of disease. The transmission stages represent major parasite population bottlenecks, and as such are ideal points of intervention for malaria eradication. A phenomenon that may be exploited for such intervention is the translational repression of specific transcripts, in which genes are proactively transcribed and selectively blocked from translation. These mRNAs are stored in membraneless cytosolic messenger ribonucleoprotein (mRNP) granules, until transmission occurs and translational repression is relieved. RNA-binding proteins of the DOZI/CITH/ALBA complex localize to distinct cytosolic mRNP granules in both asexual and sexual stage parasites. However, this complex is only known to be functionally important for translational repression in female gametocytes.

Moreover, no mechanism for how this functional switch from asexual to sexual stage parasites is appreciated. Therefore, I have leveraged the complementary strengths of conventional formaldehyde crosslinking-immunoprecipitation and proximity-dependent biotinylation (BioID, APEX2) approaches coupled to LC/MS/MS to determine if the spatial organization of this complex correlates with its function. Here, I have C-terminally tagged (e.g. GFP-; BirA*::GFP-; APEX2::GFP-) two members of the DOZI/CITH/ALBA complex, PyDOZI and PyALBA4, that have been experimentally located to complexes found at the 5' and 3' ends of mRNAs, respectively. This strategy enables the determination of short- and long-range interactions, from which we can infer changes in the subcellular organization of proteins within this complex, and whether the extension/compaction of mRNA correlates with translational status in asexual blood stage parasites and gametocytes.

43: Electron microscopy and cross-linking mass spectrometry studies of RNA editing complexes in T. brucei

***Wang, Hong (Boston University)**; Havugimana, Pierre (Departments of Biology and Biochemistry, Boston University); Huang, Lan (3Department of Physiology & Biophysics, School of Medicine, University of California, Irvine); Emili, Andrew (2Departments of Biology and Biochemistry, Boston University); Zhou, Z. Hong (4California NanoSystems Institute (CNSI), University of California, Los Angeles); Afasizhev, Ruslan (Department of Molecular and Cell Biology, Boston University School of Dental Medicine)*

Uridine insertion/deletion editing is an essential mitochondrial RNA processing pathway in kinetoplastid protists. RNA editing is mediated by trans-acting guide RNAs and often adds and deletes hundreds of uridines to generate protein coding sequences. Two principal multiprotein complexes, RNA editing core (RECC) and RNA editing substrate binding (RESC), and other accessory factors are responsible for catalyzing and regulating editing reactions. Previous studies identified a network of RNA-mediated co-complex and intra-subunit protein-protein interactions. However, the molecular architecture and mechanism of RNA recognition by RESC and assembly of the RNA editing holoenzyme remain unclear. Here, we combined cryo-electron microscopy (Cryo-EM) and cross-linking mass spectrometry (CXMS) to explore architecture of RECC and RESC complexes. Three isoforms of RECC complex and a guide RNA binding module from the RESC complex (GRBC) have been purified with rapid affinity purification and partially reconstituted in vitro. We identified inter- and intra-protein crosslinks within and between these complexes to build a low-resolution editosome model. Furthermore, we obtained cryo-EM structure of GRBC1/2 guide RNA binding heterotetramer, which is the core unit of the GRBC complex. This structure provides insights into the mechanisms of guide RNA stabilization and participation in RNA editing.

44: Mechanism of action of a novel anti-trypanosomal compound class identified from phenotypic screen

Palkar, Rima (Novartis Institutes for Biomedical Research)*; Gould, Matthew (University of Glasgow); Johnson, Nila; Ritchie, Ryan (University of Glasgow); Schmidt, Remo (Swiss TPH); Kaiser, Marcel (Swiss TPH); Maeser, Pascal (Swiss TPH); Jiricek, Jan (Novartis Institutes for Biomedical Research); Diagana, Thierry (Novartis Institutes for Biomedical Research); Barrett, Michael (University of Glasgow); Rao, Srinivasa (Novartis Institutes for Biomedical Research)*

Mechanism of action of a novel anti-trypanosomal compound class identified from phenotypic screenRima Palkar, Matthew K. Gould, Nila Johnson, Ryan Ritchie, Remo Schmidt, Marcel Kaiser, Pascal Maeser, Jan Jiricek, Thierry Diagana, Michael P Barrett and Srinivasa P S RaoWhole cell based phenotypic screening of ~2.5 million compound library against African trypanosomes identified a number of novel structural classes with potent growth inhibition. The “C-Series” structural class had low nanomolar activity in vitro against Trypanosoma brucei brucei, T. b. gambiense and T. b. rhodesiense bloodstream forms (BSFs). These compounds showed concentration and time dependent killing with an ability to sterilize trypanosomes in a short period of time. Further, C-series compounds cured both Stage I and Stage II mice models of African Trypanosomiasis.Multiple approaches such as morphological characterization, genomic and metabolomics studies were carried out to decipher the mechanism of action of C-series compounds. Phenotypic characterization using microscopy and FACS analysis using C-series inhibitors showed no effect on replication of kinetoplastid DNA but nuclear DNA replication/segregation was significantly affected. RNAseq analysis using C-series compounds showed modulation of DNA binding and nucleic acid binding proteins. Proteins involved in DNA replication such as helicase, DNA primase, DNA polymerase were down-regulated suggesting C-series compounds could be affecting this process. Untargeted metabolomics uncovered significant increases in metabolites from the nucleotide and sphingolipid pathways in C-Series exposed BSF T. b. brucei. GammaH2A phosphorylation assays show treatment with C-series compounds result in activation of DNA repair mechanisms. Future studies are in progress to narrow down on the target by using whole genome sequencing of resistant mutants and genome-wide RNAi screens. Taken together, these data suggest that C-series inhibit the nuclear DNA replication/segregation process in Trypanosomes.

45: Identification of novel druggable targets in Plasmodium falciparum using chemical mutagenesis

Clements, Rebecca L. (Harvard University); *Streva, Vincent (Boston Children's Hospital Division of Infectious Diseases); Dumoulin, Peter (Harvard T.H. Chan School of Public Health); Huang, Weigang (University of North Carolina Eshelman School of Pharmacy); Burleigh, Barbara (Harvard T.H. Chan School of Public Health); Zhang, Qisheng (University of North Carolina Eshelman School of Pharmacy); Dvorin, Jeffrey (Boston Children's Hospital Division of Infectious Diseases)*

Malaria is a deadly disease caused by Plasmodium parasites. Rapidly emerging resistance to first-line antimalarials imposes an urgent need for novel therapeutics. Our group developed a nano-luciferase reporter in Plasmodium falciparum that allows us to monitor parasite viability via luminescence intensity. Using this reporter system, we identified a compound, BCH070, that inhibits growth of P. falciparum asexual stage parasites and Trypanosoma cruzi amastigotes with an EC50 of ~100-200 nM. BCH070 also inhibited asexual growth of a panel of antimalarial-resistant P. falciparum strains, suggesting that BCH070 may act via a novel mechanism of action. BCH070 is most effective against ring-stage P. falciparum and causes rings to remain dormant up to 48 hours after the compound has been washed out. To identify the target(s) of BCH070, we selected for BCH070-resistant parasites after chemical mutagenesis with N-ethyl-N-nitrosourea (ENU). These strains demonstrate a 3-10-fold increase in EC50 compared to the parental strain. We performed whole genome sequencing on clonal parasites to identify putative targets of BCH070 and will use CRISPR/Cas to confirm that these candidates are bona fide targets. The long-term goal of this work is to identify and characterize a novel class of antimalarials and to demonstrate the potential of chemical mutagenesis to aid in target identification in Plasmodium falciparum parasites.

46: Toxoplasma effector GRA15 enhances inflammasome induced cell death in human primary fibroblasts

Mukhopadhyay, Debanjan (University of California, Davis); *Sangare, Lamba Omar (University of California, Davis); Saeij, Jeroen (University of California, Davis)*

Toxoplasma gondii is an obligate intracellular parasite that can form lifelong chronic infections in a large number of warm-blooded animals, including ~30% of humans. Interferon gamma (IFNγ) induces a variety of toxoplasmacidal effector mechanisms in human cells, which can vary between different cell types. Human fibroblasts are an important cell type for infection and persistence by Toxoplasma. Although it is known that IFNγ-stimulated human foreskin fibroblasts (HFFs) display cell death upon Toxoplasma infection, the mechanism of cell death is unknown. We used small molecule inhibitors and CRISPR/Cas9-mediated gene knockouts to explore the involvement of inflammasome driven cell death in HFFs. We found that the cell death of IFNγ-stimulated infected HFFs is dependent on Caspase 4 and Gasdermin D (GSDMD). Furthermore, we observed that the Toxoplasma dense granule protein GRA15 acts as a susceptibility factor for type II Toxoplasma strains in the IFNγ-mediated growth restriction of parasites by inducing host cell death. GRA15 knockout parasites are resistant to IFNγ-mediated growth inhibition and induce significantly less host cell death. Thus, our study shows that in human cells inflammasome activation plays an important role in mediating growth restriction of Toxoplasma.

47: Unravelling the mode of action of a novel class of antimalarials

de Vries, Laura (Radboud University Medical Centre)*; Kooij, Taco WA (Radboud University Medical Centre, Nijmegen, Netherlands); Verhoef, Julie (Radboud University Medical Centre); Allman, Erik (The Pennsylvania State University); Jansen, Patrick (Radboud University Medical Centre); Koolen, Karin (TropIQ Health Sciences); Bolscher, Judith (TropIQ Health Sciences); Vos, Martijn (TropIQ Health Sciences); Josling, Gabrielle (The Pennsylvania State University); Llinás, Manuel (The Pennsylvania State University); Sauerwein, Robert (Radboud University Medical Centre); Schalkwijk, Joost (Radboud University Medical Centre); Dechering, Koen (TropIQ Health Sciences)*

Due to the emerging resistance against all front-line antimalarials, there is an urgent need for development of drugs that target novel pathways and affect multiple stages of the parasite. Malaria parasites strongly rely on the extracellular supply of pantothenate (vitamin B5) and synthesized pantothenate derivatives (pantothenamides) are active against Plasmodium falciparum asexual and sexual blood stages at nanomolar concentrations. Metabolomic analyses of pantothenamide-treated parasites showed a significant reduction in phosphopantothenate and acetyl-CoA. Furthermore, the identification ofdrug-derived CoA analogues demonstrated that pantothenamides are processed. Whole-genome sequencing of two independent, pantothenamide-induced, resistant parasite lines revealed mutations in the binding pockets of two CoA-binding enzymes. Both lines revealed the same mutation in acetyl-CoA synthetase (ACS) and a second mutation in acyl-CoA synthetase 11 (ACS11) though on different amino acids. Using CRISPR-Cas9, we first introduced the conserved point mutation in ACS in NF54 parasites. Subsequently, we introduced either of the ACS11 mutations. In comparison to wild-type parasites, IC50 values were 15 fold higher for the engineered ACS single mutant and at least 94 fold higher for all four double mutants, both genetically modified and drug-selected. Furthermore, all lines were cross-resistant to at least three different pantothenamides, suggesting a similar mechanism of action for these compounds. Conversely, reversion of the point mutations back to the wild-type sequences in the drug-selected parasites restored drug sensitivity. Currently, we are exploring the resistance phenotype and mechanism of action of pantothenamides during transmission by generating the single and double mutants in a GFP-Luc reporter line. Overall, we have synthesized potent new multistage antimalarials that target a novel pathway. Our data suggest that drug-derived CoA analogues may act as antimetabolites that target acetyl-CoA synthetase and thereby inhibit the conversion of CoA to acetyl-CoA.

48: Deciphering STEVOR adhesive properties in Plasmodium falciparum gametocyte-infected erythrocytes

Neveu, Gaëlle (INSERM); *dupuy, florian (INSERM); Ladli, Meriem (INSERM); Barbieri, Daniela (INSERM); Naissant, Bernina (INSERM); Richard, Cyrielle (INSERM); Bachmann, Anna (Bernhard Nocht Institute for Tropical Medicine); Bischoff, Emmanuel (Institut Pasteur); Verdier, Frédérique (INSERM); Lavazec, Catherine (INSERM)*

Plasmodium falciparum gametocytes, the only parasite stages responsible for transmission from human to mosquitoes, have been recently shown to sequester and develop in the human bone marrow parenchyma, where they are enriched at erythroblastic islands. These specialized niches, where occurs the terminal erythroid differentiation, consist of a macrophage surrounded by differentiating erythroblasts. The presence of gametocytes in this niche suggests that gametocyte-infected erythrocytes may adhere to erythroid cells. In asexual stages, STEVORs function as erythrocyte-binding proteins that recognize Glycophorin C (GPC) on the surface of uninfected erythrocytes.

However, STEVORs adhesive properties have never been investigated during sexual stages, where their interaction with GPC located at the surface of erythroid progenitors is plausible since GPC protein expression starts in the early stage of erythropoiesis. To address this hypothesis, we have performed cell-cell adhesion assays to determine whether gametocyte-infected erythrocytes adhere to human primary erythroblasts or erythroid cell lines. In order to determine the contribution of STEVOR proteins to these interactions, we used clonal lines that express specific stevor genes as well as parasite lines that overexpress or down-regulate members of the stevor gene family. Adhesion assays indicate that gametocyte-infected erythrocytes do not specifically adhere to erythroblasts, even when the parasite adhesins STEVOR are overexpressed at the infected erythrocyte membrane.

49: Protozoa are a global burden - and need new treatments and novel drug targets

Mbekeani, Alison (University of Durham)

Protozoa are a global burden - and need new treatments and novel drug targets:
1. Investigating Toxoplasma gondii peroxisomes; are they druggable?
2. Exploration of natural products as anti-leishmanials and anti-toxoplasmosis agents The use of natural products (NP) for treating protozoan infections could be dated back to 1631 in Rome, where cinchona tree bark was used to cure malaria. The discovery of new NP, in the treatment of protozoan diseases is absolutely vital for many of these vaccine deficient protozoan infections. The assessment of NP against Cutaneous Leishmaniasis, was explored using a library of NP screened against the mammalian stage of L. Mexicana in in vitro assays. In addition we also explored the use of a NP, Aureobasidin A and its five derivatives against T. gondii Type I and Type II. Investigating a drug target in T. gondii, we focused on the presence of peroxisomes within T. gondii using Pex proteins. The experimental approach taken involved characterization of putative TgPex5 and it's associated putative protein ligand TgSCP2. TgSCP2 with a C-terminal Peroxisomal Targeting Signal 1 (PTS1) binds TgPex5, whilst the N-terminal of TgPex5 binds TgPex7. Using molecular biology, reverse genetics and protein characterization, we show that pull-down assays, localisation of TgSCP2, and complementation of TgPex5 and TgPex7 in yeast and human expression systems, we are able to compile evidence to prove or refute the presence of peroxisomes within T. gondii.

50: Characterization of multiple MORN-repeat proteins in Toxoplasma gondii and their potential roles in phosphoinositide/calcium signalling

Ke, Huiling (University Of Cambridge); *Koreny, Ludek; Barylyuk, Konstantin; Waller, Ross*

Apicomplexan parasite Toxoplasma gondii infects about one-third of the global human population. Toxoplasmosis is a life-threatening disease in immunocompromised individuals and a significant cause of miscarriage and/or severe disorders in newborns. Apicomplexan parasites use calcium as a messenger to mediate many essential intracellular processes including microneme secretion and gliding motility, both of which are integral to host invasion and egress. Calcium release from intracellular stores in parasites is thought to be induced by regulated inositol (1,4,5)-trisphosphate (IP3) generated from phosphoinositide (PI) metabolism. In plants, a phosphatidylinositol monophosphate kinase (PIPK) is linked to a membrane occupation and recognition nexus (MORN) domain for membrane association of this enzyme. While one Toxoplasma MORN protein (TgMORN1) has been characterised in some detail, we note that there are four other TgMORN proteins. Spatial proteomic data generated by our lab indicates that these MORN proteins are associated with PI metabolic enzymes (e.g. PIPK, PIP phosphatase and cyclic nucleotide phosphodiesterase), suggesting that MORN domains might participate in the recruitment of these enzymes and control of PI/Ca2+-based signalling, perhaps similarly to plants. In this study, the subcellular localizations of several MORN-repeat proteins have been determined, some of which showed co-localization with calcium signalling-related proteins. Conditional knock-down mutants of these MORN proteins have been generated and the phenotypic effects of protein depletion are being analysed, including the effects on the locations of PI metabolic enzymes. This work will test for a relationship between MORN-repeat proteins and invasion-related signalling events, which helps to extend the knowledge of the pathogenesis of T. gondii.

51: Transcription initiation defines kinetoplast RNA boundaries

Suematsu, Takuma (Boston University); *Sement, François M. (Boston University); Yu, Tian (Boston University); Zhang, Liye (ShanghaiTech University); Huang, Lan (University of California, Irvine); Afasizheva, Inna (Boston University); Afasizhev, Ruslan (Boston University)*

Mitochondrial genomes are often transcribed into long primary RNAs punctuated by tRNAs whose excision defines RNA boundaries. In T. brucei, kinetoplast DNA is composed of ~23-kb maxicircles encoding two rRNAs and 18 protein genes, and ~1-kb minicircles encoding guide RNAs. Although kDNA lacks tRNA genes, it is commonly held that maxicircle transcription is polycistronic while the precursor is partitioned into pre-mRNAs by a “cryptic” endonuclease. Recently, we established mitochondrial 3′ processome (MPsome) as the key processing complex acting on primary transcripts. We also demonstrated that MPsome-catalyzed 3′-5′ degradation defines mature mRNA, rRNA and gRNAs 3′ ends. It follows that such mechanism is incongruent with a polycistronic precursor structure and “endonuclease model”. However, a plausible pathway involving exonuclease must account for the monophosphorylated 5′ and homogenous 3′ termini observed in steady-state RNA pool. Here, we show that individual mRNAs and rRNAs are independently synthesized as 3′ extended precursors. The transcription-defined 5′ terminus is converted into monophosphorylated state by the 5′ pyrophosphohydrolase complex. This novel particle, termed PPsome, is composed of MERS1 NUDIX hydrolase and sequence-specific RNA binding subunit. PPsome is targeted to 5′ ends of all mRNAs and is activated by the RNA editing substrate binding complex. Most gRNAs lack PPsome recognition sites and, therefore, remain triphosphorylated. We provide evidence that both 5′ pyrophosphate removal and 3′ adenylation are essential for mRNA stabilization and uncover a mechanism by which non-coding antisense RNAs control 3′-5′ exonucleolytic degradation. We conclude that mRNAs and rRNAs are transcribed and processed as insulated units irrespective of their genomic location. It is conceivable that transcriptional control plays a significant role in developmental regulation of mitochondrial gene expression.

52: Localization and role of a mechanosensitive channel in the procyclic form of Trypanosoma brucei

Hernandez, Monica (California State University Fullerton)*; Nguyen, Kristy; Dave, Noopur; Jimenez, Veronica (California State University Fullerton)*

Trypanosoma brucei (T. brucei) is a protozoan parasite transmitted by the tsetse fly and the causative agent of African Sleeping Sickness. Once the parasite has established infection in the host it can pass the blood brain barrier causing psychological and neurological disturbances. At this stage, the mortality rate is close to 100%. Current treatment causes severe side effects and often require prolonged intravenous administration, highlighting the need for new therapeutic options. Potential targets include mechanosensitive channels (MSCs) which have been shown to contribute to bacterial virulency. Screening of T. brucei's genome revealed a putative mechanosensitive channel, TbMscS. TbMscS shares 64% identity with a recently characterized MscS-like channel in T. cruzi and 31% identity with E. coli MscS. Preliminary topology reveals the channel has three transmembrane domains, similar to the E. coli channel. To establish the localization of the channel, we have incorporated a myc tag at the C-terminal end of the endogenous locus. Our findings indicate the protein is localized along the plasma membrane of the parasite suggesting that the channel could play a role in detection of extracellular signals and activation of intracellular pathways during mechanical and physiological stress. Upon knockdown of the channel the parasites show no significant growth defects but their ability to maintain their nomal volume is severely affected under hyperosmotic conditions. Our results suggest that TbMscS is a mechanosensitive channel important for regulating physiological responses in the procyclic form of T. brucei. Since bacterial-like MscS channels are not present in humans, TbMscS could be a promising drug target against an economic and medical burdensome disease.

53: Analysis of mechanosensation-dependent functions in Trypanosoma cruzi

Fonbuena, Joshua (California State University Fullerton)*; Feldman, Marc; Tiwari, Megna; Jimenez, Veronica (California State University Fullerton)*

Trypanosoma cruzi propagates from an insect vector to a mammalian host to complete its life cycle. To cope with the changing conditions in these environments, the parasite has developed robust compensatory mechanisms; however, the sensory machinery utilized to detect variations in the extracellular and intracellular conditions remains unknown. In all cell types, mechanosensation is responsible for sensing and responding to changes in pressure, osmolarity, and tension of the membrane. In addition, in bacteria mechanosensation is associated with virulence-related traits such as biofilm formation and quorum sensing. In T. cruzi, we have identified and characterized a mechanosensitive channel (TcMscS) that shares structural and functional features with the small conductance mechanosensitive channel, MscS of E. coli. TcMscS is differentially localized in the three main life stages of the parasite with the extracellular forms expressing the channel in the contractile vacuole, while intracellular amastigotes show it distributed in the plasma membrane. Gene knockout by CRISPR-Cas9 severely reduced the growth of extracellular and intracellular parasites and decreased the ability to compensate osmotic challenges in epimastigotes. RNaseq analysis in TcMscS-KO parasites shows changes in gene expression of several channels and transporters, supporting the hypothesis that ion homeostasis is maintained by a network of interconnected proteins. Our results indicate that mechanosensitive-activated channels are part of the physiological responses that allow the parasites to differentiate and effectively infect host cells and could represent a potential drug target against T. cruzi.

54: Role of a Secreted Effector of Toxoplasma gondii in Modulating the Host Cell Cycle

Pierre-Louis, Edwin (University of Georgia); *Etheridge, Menna (University of Georgia); Etheridge, R. Drew (University of Georgia)*

Toxoplasma gondii is arguably one of the most successful parasitic protozoans on the planet. It is estimated that T. gondii infects approximately a third of humanity and nearly half of all warm-blooded animals. Upon invasion of its host cell, Toxoplasma induces a massive reorganization of the host cell transcriptional program and, as a result, major pathways related to metabolism, immunity and the cell cycle become activated. T. gondiihas been shown to initiate modification of its host cell environment through the regulated secretion of its various secretory organelles (micronemes, rhoptries, dense granules).
The dense granules have emerged as an important reservoir of various host nuclear targeted parasite effectors with diverse targets and functions. In our preliminary studies, we have identified a dense granule protein that translocates across the parasitophorous vacuole membrane, traffics to the host nucleus and is responsible, in part, for the modulation of the host cell cycle. As a result, we have since named this protein ICC1 for inducer of the cell cycle protein 1. We have demonstrated that ICC1 is responsible for modulating the expression of a multitude of host genes involved in progression of the host cell cycle into S-phase. Parasites lacking this protein demonstrated a significant kinetic delay in their ability to induce host cell cycle transition and an altered plaquing phenotype. We will discuss the unique effects of ICC1 on host cell cycle progression and highlight evidence of a second secreted parasite effector operating with slower kinetics but also with the ability to induce a G1/S-phase transition in the host.

55: Mutational analysis of the leucine zipper in APOL1 and the effect on channel formation

Lee, Penny (CUNY Hunter College)*; Schaub, Charles (CUNY Hunter College); Racho-Jansen, Alisha (CUNY Hunter College); Kim, Ryan; Raper, Jayne (CUNY Hunter College)*

African Trypanosomes are unicellular, flagellated parasites transmitted through tsetse flies in sub-Saharan Africa. Humans are not prone to infection by Trypanosoma brucei brucei due to a subset of high density lipoprotein (HDL) termed Trypanosome Lytic Factor (TLF). Thelytic component of TLF, Apolipoprotein L-1 (APOL1), is a pH-gated cation channel that causes swelling and lysis of the parasites. When TLFs are taken up by the trypanosomes into the acidic endosome, APOL1 dissociates from the TLF complex, which is necessary for APOL1 to insertinto the endosomal membrane. APOL1 is then recycled back to the plasma membrane where the neutral pH of the extracellular space opens the channel resulting in osmotic imbalance, an influx of water and subsequent lysis. To understand the mechanism of channel formation, we are investigating the structural domains of APOL1. The C-terminus contains a putative leucine zipper motif, which is potentially responsible for oligomerization. Through the purification of recombinant APOL1 (rAPOL1) derived from E. coli, we are able to demonstrate the effects of single and multiple amino acid mutations with in vitro trypanolysis assays.
Furthermore, the ability of these mutants to form oligomers was tested in biochemical and electrophysiological assays. Altogether, the data suggests APOL1 is an oligomer as a functional channel.

56: Identifying an APOLI Binding Partner Through SRA-Specific Antibodies

Ko, Daphne (CUNY Hunter College)*; Verdi, Joey (CUNY Hunter College); Leidich, Raymond; Huang, Tammy; Raper, Jayne (CUNY Hunter College)*

African trypanosomes are unicellular parasites that cause African trypanosomiasis. Trypanosomes take up trypanosome lytic factors (TLF), a subset of high-density lipoproteins (HDLs), that allow humans to resist infection by Trypanosoma brucei brucei. TLFs contain Apolipoprotein L-I (APOLI), which forms cation channels in trypanosomes, resulting in osmotic imbalance followed by trypanolysis. However, human APOLI cannot confer resistance to T.b. rhodesiense because the parasite has serum resistance associated protein (SRA), which binds to APOLI and prevents channel formation. This interaction can be blocked with antibodies specific for the APOLI-binding region on SRA. These SRA antibodies were tested in vitro with trypanolysis assays in the presence of HDL or whole serum and in vivo with APOLI (G0)-expressing mice. The antibodies enhanced killing of SRA-expressing trypanosomes in the presence of HDL, but not in whole human serum or in mice. This suggests that there may be an SRA-like APOLI binding partner in serum that prevents G0 APOLI from forming channels in host cells. As opposed to the SRA-binding G0 APOLI, there exist variants in African and African American populations, G1 and G2, which confer resistance to T.b. rhodesiense due to amino acid changes in the SRA-binding region. However, two copies of G1 or G2 APOLI also lead to higher risk of kidney disease. We propose the presence of an APOLI binding partner, similar to the trypanosome-specific SRA, that can bind G0 and prevent channel formation in host cells, but not APOL1 G1 and G2. This lack of protection increases risk of kidney disease. Through affinity chromatography with SRA antibodies, this binding partner can be identified and sequenced.

57: The Promiscuous IgM Antibodies Associated with Trypanosome Lytic Factors

Verdi, Joey (Hunter College, City University of New York)*; Zipkin, Ronnie (Hunter College, City University of New York); Sternberg, Jeremy (University of Aberdeen); Thomson, Russell (Hunter College, City University of New York); Raper, Jayne (Hunter College, City University of New York)*

Human immunity to infection by Trypanosoma brucei brucei is mediated by two lipoprotein complexes called trypanosome lytic factors (TLFs) 1 and 2. The unique components of TLFs are the pore-forming protein apolipoprotein L-I (APOLI) and hemoglobin (Hb) binding protein haptoglobin-related protein (HPR). HPR-Hb complexes are recognized by the trypanosome haptoglobin-hemoglobin receptor (HpHbR). Although TLF1 endocytosis is facilitated by this receptor, TLF2 endocytosis is independent of the HpHbR and is facilitated by an unknown mechanism. TLF2 is distinguished from TLF1 by the inclusion of non-covalently associated IgM antibodies that may play a role in TLF2 endocytosis. Using TLF2-associated IgMs (TLF2-IgMs) isolated from healthy donors, we find that TLF2-IgMs interact with many trypanosome antigens in vitro, suggesting that TLF2-IgMs could facilitate endocytosis by directly interacting with the trypanosome. TLF2-IgMs also interact with HPR, potentially mediating complex assembly. Taken together, these data suggest that polyreactive TLF2-IgMs may target TLF complexes to the trypanosome by interacting with the trypanosome and HPR simultaneously. Since HPR is found on all TLFs, we hypothesized that TLF2-IgMs could interact with TLF1 complexes to form TLF2 in an equilibrium reaction dependent on the concentration of TLF2-IgMs. IgM concentrations increase during trypanosome infections and we find that serum samples from trypanosome infected human patients show a higher ratio of TLF2 to TLF1 complexes than uninfected controls. These data suggest that the immune response to trypanosomes increases the production of TLF2-IgMs in vivo, leading to an increase in TLF2 complexes that interact with the trypanosome surface in an IgM dependent manner.

58: Spatial organization of the blood stage parasitophorous vacuole of Plasmodium falciparum

***Garten, Matthias**; Beck, Josh R; Glushakova, Svetlana; Bleck, Christopher K.E.; Heuser, John; Tenkova-Heuser, Tatyana; Roth, Robyn; Zimmerberg, Joshua; Goldberg, Daniel*

Blood stage *Plasmodium spp.* thrive inside the parasitophorous vacuole (PV). The PV membrane (PVM) is the interface separating the parasite-controlled microenvironment from the red blood cell (RBC) cytosol. To export proteins and waste products, and to import nutrients, the parasite relies on transport across the PVM. Curiously, PVM resident proteins organize into discrete patches. Similarly, the PV lumen (space between the PVM and the parasite plasma membrane, PPM) is variable, seen as “beads on a string” in cross-sections.
Here we hypothesized that the PV lumen and PVM proteins are coordinated, presumably to facilitate the various functions of the PVM, i.e. create district sites for protein export, nutrient import and hemoglobin uptake. Using differential fluorescent labeling of the PVM and the PV lumen, with and without protein export block, we found that EXP2 and regions of detectable PV lumen are apparently colocalized in the trophozoite stage. This PV lumenal space was not uniform, and enlarged significantly upon blockage of protein export. We interpret this result as evidence for the existence of EXP2 depleted, PVM-PPM attachment sites. The observation of two distinct PV zones is substantiated by freeze-fracture replica analysis by electron microscopy, which revealed a PVM segregated into two domains: one domain both rich in intramembraneous particles (IMP) and EXP2-immunostain positive, and a second domain completely free of IMP.
This is one of the first demonstrations of membrane domain formation in biology on the basis of IMP inclusion or exclusion. Understanding the mechanism of domain formation and its physiological role may open the door to connect a large biophysical literature on membrane domains to the medical problems of parasite infections.

59: Increased expression glucosylceramide transferase in Giardia interferes with cyst production and parasite infectivity

Enriquez, Vanessa (Department of Biological Sciences, University of Texas at El Paso, El Paso, TX)*; Grajeda, Brian (Department of Biological Sciences, University of Texas at El Paso, El Paso, TX); De Chatterjee, Atasi (Department of Biological Sciences, University of Texas at El Paso, El Paso, TX); Diaz-Martinez, Laura (Department of Biological Sciences, University of Texas at El Paso, El Paso, TX); Das, Siddhartha (Department of Biological Sciences, University of Texas at El Paso, El Paso, TX)*

Background:Giardia lamblia, a waterborne parasite, is responsible for the intestinal infection Giardiasis in both developed and developing countries. Giardiasis is transmitted via infective cysts through contaminated food and water. We have reported earlier that giardial glucosylceramide transferase 1 (gGlcT1), an enzyme of sphingolipid metabolism, is involved in regulating encystation (cyst production) and that interrupting its activity affects the biogenesis of encystation-specific vesicles (ESVs), cyst morphology, and viability. Goal: The goal of this study is to modulate the expression of GlcT1 in Giardia and evaluate its role in growth, encystation, and infection. Strategy and methods: Three different gGlcT1 expression plasmids were constructed and used in the current study and they are: (1) an endogenously tagged gGlcT1-HA plasmid (endo-gGlcT1-HA); (2) an overexpression plasmid containing a weak a-tubulin promoter (aTub-gGlcT1-AU1) and (3) a plasmid under a strong giardial OCT (ornithine carbamoyltransferase)-promoter (OCT-gGlcT1-HA). Expressions of gGlcT1 by individual clones were monitored by immunoblots. Encystation/cyst production was evaluated by immunofluorescent microscopy and infectivity was tested in C57BL/6 mice. Results and conclusion: While cells transfected with endo-gGlcT1-HA and aTub-gGlcT1-AU1 showed similar encystation-induced expression patterns, OCT-gGlcT1-HA exhibited a different profile. OCT-gGlcT1-HA cells constitutively expressed the protein (i.e., gGlcT1) in trophozoites and encysting cells but the expression reduced dramatically in cysts. The overall growth of OCT-gGlcT1-HA trophozoites was also low. In vitro-derived cysts of OCT-gGlcT1-HA cells appeared mostly as type II rather than type I. Both aTub-gGlcT1-AU1 and OCT-gGlcT1-HA Giardia showed reduced or delayed infectivity in mice compared to the non-gGlcT1 transfected parasite. These results suggest that the regulated expression of gGlcT1 is critical for the growth, encystation and infection by Giardia.

60: Characterizing Genes Involved in Trogocytosis (cell-nibbling) in Entamoeba histolytica

Feeney, Shea, E. (University of California, Davis); *Ralston, Katherine, S. (University of California, Davis)*

Entamoeba histolytica (histo-: tissue; lytic-: dissolving) is a eukaryotic pathogen that causes amoebiasis in humans—a significant global burden with about 50 million diarrheal infections and 100,000 deaths per year. E. histolytica's ability to cause profound tissue damage is likely a result of a mechanism by which amoebae kill human cells by performing trogocytosis (trogo-: nibble), or taking 'nibbles', of living cells leading to cell death. In contrast, amoebae engulf dead (pre-killed) human cells whole via phagocytosis (phago-: devour). While there are some commonalties between trogocytosis and phagocytosis, we hypothesize that amoebic trogocytosis requires genes that are distinct from phagocytosis. There is evidence that phagocytosis is under feed-forward gene regulation in E. histolytica. We have shown that amoebae that are exposed to living human cells perform more trogocytosis after a second round of exposure to living human cells, suggesting that trogocytosis is likewise under feed-forward gene regulation. To identify genes differentially involved in trogocytosis versus phagocytosis, E. histolytica were fed live or dead human cells, respectively, to prompt genes that are involved in these processes to be up-regulated. We performed RNA-seq analysis to identify genes that changed substantially in comparison to the reference RNA from amoebae that have not been fed human cells. Over 1000 genes were found to be significantly differentially expressed between trogocytosis and phagocytosis and were involved in various processes including cytoskeletal rearrangement. To further validate, several uncovered putative trogocytosis genes will be knocked-down and evaluated for a trogocytosis defect. Since trogocytosis appears to be a fundamental form of eukaryotic cell-cell interaction yet is exploited for cell killing by certain microbes, this work should yield broad insight.

61: Developing a Genome-wide RNAi Knock Down Screen in Entamoeba histolytica

Bettadapur, Akhila (University of California, Davis); *Ralston, Katherine S. (University of California, Davis)*

Entamoeba histolytica is a microbial eukaryote and causative agent of the disease amoebiasis. Pathogenesis is associated with a profound damage to human tissues, and is likely caused by E. histolytica cells (“amoebae”) killing human cells through the novel cell-nibbling process named trogocytosis (trogo-: nibble). Efforts to systematically uncover genes involved in understudied mechanisms are hampered by the lack of available forward genetic tools.
Building on existing tools that utilize endogenous RNA interference (RNAi) silencing machinery, we aim to perform the first forward genetic screen in E.histolytica by constructing a genome-wide RNAi knock down library. Using an E. histolytica expression plasmid, we are creating a library of knock down plasmids by cloning genomic DNA adjacent to a silenced gene that has endogenously expressed small RNAs. Through spreading of silencing from the silenced gene, small RNAs are generated to the genomic DNA fragment, leading to RNAi knock down of the corresponding gene. To aid in calculating transfected library coverage and efficiency, we have determined that although the forward directionality of the insert matters, both in frame and out of frame genomic DNA inserts lead to knockdown. Additionally, we have determined stable transfection efficiency of the knock down plasmids to be approximately one in 1000. This library will have many potential future applications, as it is an effective and unbiased tool to identify genes involved in many different processes. This includes distinguishing essential genes from those that can be knocked down, as well as understanding the potential avenues of resistance to drugs. While greatly expanding genetic tools in E. histolytica, this novel screen will improve understanding of amoebiasis pathogenesis as a whole.

62: Epistasis studies reveal functional redundancy among calcium-dependent protein kinases in motility and invasion of malaria parasites

Fang, Hanwei (University of Geneva); *Gomes, Ana Rita (University of Montpellier); Klages, Natacha (University of Geneva); Pino, Paco (University of Geneva); Maco, Bohumil (University of Geneva); Walker, Eloise (London School of Hygiene and Tropical Medicine); Zenonos, Zenon (Wellcome Trust Sanger Institute); Angrisano, Fiona (Imperial College London); Baum, Jake (Imperial College London); Doerig, Christian (Monash University); Baker, David (London School of Hygiene and Tropical Medicine); Billker, Oliver (Wellcome Trust Sanger Institute); Brochet, Mathieu (University of Geneva)*

In malaria parasites, evolution of parasitism has been linked to functional optimisation necessitated by genomic reductions. Despite this optimisation, most members of a calcium-dependent protein kinase (CDPK) family show genetic redundancy during erythrocytic proliferation. To identify relationships between phospho-signalling pathways, we screened for 294 genetic interactions among protein kinases in Plasmodium berghei. We identified a synthetic negative interaction between a hypomorphic allele of the cGMP-dependent protein kinase G and CDPK4 to control erythrocyte invasion which is conserved in P. falciparum. CDPK4 activity becomes critical when PKG-dependent calcium signals are attenuated to phosphorylate two proteins important for the stability of the inner membrane complex, which serves as an anchor for the acto-myosin motor required for motility and invasion. Finally, we show that multiple kinases including CDPK1 can functionally complement CDPK4 during erythrocytic proliferation and transmission to the mosquito vector. Altogether, this study reveals how CDPKs are functionally wired within a stage-transcending signalling network to control motility and host cell invasion in malaria parasites.

63: Systematic Identification of host factors that regulate Plasmodium LS infection

Probst, Alexandra S. (Center for Infectious Disease Research)*; Lewis, Adam (Center for Infectious Disease Research); Vijayan, Kamalakannan (Center for Infectious Disease Research); Parks, K. Rachael (Fred Hutchinson Cancer Research Center); Arang, Nadia (University of California, San Diego); Kaushansky, Alexis (Center for Infectious Disease Research)*

The liver stage of malaria infection is the first obligate stage within the mammalian host, but host-pathogen interactions between the transmission form of the parasite, the sporozoite, and the hepatocyte remain largely undescribed. We and others have previously elucidated the critical role of multiple hepatocyte receptors in Plasmodium infection, including the receptor tyrosine kinase EphA2. Interestingly, the knockout of EphA2 does not entirely inhibit hepatocyte infection. To identify additional factors that facilitate Plasmodium LS infection, we generated a genome-wide CRISPR/Cas9 knockout (GeCKO) hepatoma cell library and infected with GFP-expressing P. yoelii sporozoites. Uninfected and infected cells were sorted by fluorescence activated cell sorting and sequenced to identify gene knockouts that had significant impact on infection. Small guide RNAs (sgRNAs) directed against several hepatocytes genes involved in the p53 signaling pathway, melanogenesis and circadian entrainment were found to be differentially expressed in infected cells. Additionally, sgRNAs against several host receptors exhibited decreased levels within infected cells, including multiple members of the Eph family of Receptor Tyrosine kinases. Specifically, two separate sgRNAs directed against EphB4, and one guide against EphA1 were significantly reduced the P. yoelii-infected cells, suggesting that the absence of one or more Eph receptor diminishes infection. Ongoing work is focused on elucidating the molecular role that multiple host receptors play in Plasmodium liver stage infection.

64: ERAD and disposal of GPI-Anchored proteins in African trypanosomes

Bangs, James D. (Department of Microbiology & Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo (SUNY), Buffalo, NY, 14214, USA.)*; Koeller, Carolina M. (Department of Microbiology & Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo (SUNY), Buffalo, NY, 14214, USA.); Tiengwe, Calvin (Department of Life Sciences, Imperial College, London, UK.)*

The main mode of antigenic variation in African trypanosomes is gene conversion of the resident Variant Surface Glycoprotein (VSG) gene in the active expression site, a process that is often segmental and which will eventually lead to catastrophic generation of a misfolded glycosylphosphatidylinositol (GPI-anchored VSG. Misfolded secretory proteins are generally retro-translocated for degradation in the proteasome by ER associated degradation (ERAD). However, in yeast and mammals misfolded GPI-anchored proteins are preferentially transported to the vacuole/lysosome for disposal. This is because GPIs act as signals for forward trafficking from the ER. Trypanosomes are capable of ERAD and we have proposed this is a critical for surviving a VSG misfolding event, but only if able to handle disposal of GPI-anchored cargo. We investigate this process by RNAi silencing of endogenous transferrin receptor (TfR) while expressing a resistant misfolded TfR subunit (HA:E6). TfR is a heterodimer of GPI-anchored ESAG6 (E6) and soluble ESAG7 (E7). When expressed with ESAG7, HA:E6 heterodimerizes, but does not bind transferrin. When expressed alone, HA:E6 is N-glycosylated and GPI-anchored, but accumulates in the ER as monomer/aggregates.

Treatment with MG132, a proteasome inhibitor, generates a full-length polypeptide that is cytosolic and de-N-glycosylated (HA:E6*). HA:E6* is innately reactive with anti-CRD antibody, a GPI-specific reagent, indicating the GPI anchor is not a prerequisite for proteasomal degradation. The trypanosome GPI anchor is a forward trafficking signal, thus the dynamic tension between ERAD and ER exit favors degradation by ERAD. These results differ markedly from the standard eukaryotic model systems, and indicate that ERAD is a backup system in case of a VSG misfolding. Thus ERAD of GPI-anchored proteins may provide an evolutionary advantage related to pathogenesis in African trypanosomes.

65: Detection and isolation of Trypanosoma brucei extracellular vesicles during an active infection

Cipriano, Michael J. (University of Georgia, Athens)*; Palmer, Margot (University of Georgia, Athens); Hajduk, Steven (University of Georgia, Athens)*

Extracellular vesicles (EVs) are produced by nearly all cell types so far studied. The importance of EVs in infection has been shown for many pathogens but research in Trypanosoma brucei extracellular vesicles are only beginning to show their importance in infection and disease symptoms.While Trypanosoma brucei EVs have been shown to be produced in in-vitro cell culture, we have now detected the production of EVs during a persistent and acute infection. We can detect EV proteins in multiple bodily fluids of an infected mouse with great sensitivity by using a strain engineered to produce EV proteins fused to nanoluciferase. Additionally, we can isolate these EVs from mouse serum using standard EV isolation techniques and detect their presence using antibodies.
EV proteins can be detected at low parasitemia levels before they can be observed via standard microscopy procedures. After clearance by the immune system, EV proteins persist for many days after in the serum and urine, widening the ability to detect a prepatent infection. We have also begun to determine the role EVs play in affecting the host during an active infection.EVs provide an optimal target for detection of an infection as well as a promising area of research into determining the causes of Trypanosome disease symptoms.

66: The chromatin-bound proteome of the human malaria parasite, Plasmodium falciparum

Williams, Desiree (University of California Riverside)

Proteins interacting with DNA are known to mediate fundamental aspects of cellular processes such as gene expression, DNA replication, DNA repair and maintenance of genome integrity. Plasmodium falciparum, the causative agent of malaria develops through a complex life cycle that requires the coordinated expression of its genes. It has recently been shown that the parasite exerts limited transcriptional control of gene expression. Accumulating evidence suggests that parasite chromatin is highly structured at the three-dimensional level, and provides an epigenetic mechanism to regulate gene expression. To understand how parasite 3D nuclear structure is being maintained and regulated, we undertook complementary computational and experimental approaches to identify and characterize chromatin-associated proteins (CAPs) in P. falciparum. Over a 1000 putative CAPs were identified by hidden Markov models and NCBI RPS-BLAST searches. Several chromatin-associated domains (CADs) were enriched in apicomplexan parasites and plant species. Using a novel mass spectrometry approach that specifically enriches for CAPs, we experimentally captured 987 CAPs during the parasite erythrocytic stages. Identified CAPs include many characterized chromatin regulators such as histone-modifying enzymes, parasite-specific transcription factors, DNA repair proteins, and chromatin-assembly factors. Finally, we validated two of our candidate proteins using standard cellular and molecular approaches. One such protein, characterized as a CROWDED-like NUCLEI (CRWN)protein, is a plant protein that is functionally analogous to the animal nuclear lamina.Collectively, our results provide the most comprehensive overview of CAPs in Plasmodium. A better understanding of these CAPs will not only provide a complete picture of the complex molecular components that regulate chromatin structure and genome architecture in the parasite, but could also lead to the identification of new antimalarial strategies.

67: A secreted kinase regulates membrane structures of the Toxoplasma parasitophorous vacuole

Beraki, Tsebaot (Ut Southwestern medical center); *Hu, Xiaoyu; Young, Joanna; O'Shaughnessy, William; Treeck, Moritz; L Reese, Michael*

We recently identified a family of secreted kinases that are conserved throughout coccidia that lack the Gly-loop which is thought to be essential for ATP binding. We have therefore named this family the With No Glycine (WNG) kinases. Despite lacking a motif that is thought to be critical for catalysis, our data show that WNG kinases are indeed able to catalyze phosphoryl transfer. We solved the crystal structure of one member of the family, which revealed a non- canonical active site. Knockouts of the most conserved member of the family, WNG1/ROP35, show reduced virulence and cyst burden in a mouse model of infection. While most kinases secreted by Toxoplasma have been found to act on host molecules, the WNG kinases localize to the dense granules and the lumen of the parasitophorous vacuole (PV). Comparative phosphoproteomics between wild-type and WNG1 knockout parasites revealed that WNG1 appears to phosphorylate a number of GRA proteins that have been previously shown associated with the biogenesis of the membranous intravacuolar network (IVN). In WNG1 knockout parasites, a subset of these GRA proteins showed reduced membrane association. Consistent with these data, the IVN in WNG1 knockouts is partially disrupted, suggesting that WNG1-mediated phosphorylation regulates its biogenesis and stability.

68: Unexpected Link Between Lipid Droplets, Cell Morphology, and Glucose Metabolism

Pazzo, Kyle (Clemson University); *Paul, Kimberly (Clemson University); Raja, Sripriya (Clemson University); Featherstone, Ellen (Clemson University); Adamson, Michelle (SC Governor's School for Science and Mathematics); Croft, Lanie (SC Governor's School for Science and Mathematics)*

Lipid droplets (LDs) are dynamic organelles formed from the ER that are involved in lipid storage and metabolism. LD proteins and the roles of LDs in Trypanosoma brucei remain poorly characterized. We generated two constructs (thuPLIN-eYFP & thuPLIN-CC-eYFP) based on a human Perilipin 1 amphipathic helix sufficient to target GFP to yeast LDs. Over-expression of eYFP-tagged thuPLIN constructs in procyclic cells resulted in dramatic cellular elongation (up to ~40 μm), a halt in cell division, and eventual culture death.

Fluorescence patterns did not resemble expected LD punctate pattern, instead they showed primarily diffuse cytosolic localization, with punctate and reticular features being more prominent as drug selection proceeded. Over-expression of thuPLIN-2Xmyc also resulted in elongation and death, suggesting this morphological response is independent of epitope tag. Over-expression of T. brucei Erg6-eYFP, a cholesterol synthetic enzyme that is LD-localized in other organisms, resulted in elongation, but no elongation was observed with in situ myc-tagged Erg6 under endogenous expression levels. Unlike the thuPLIN constructs, both over- and endogenously-expressed Erg6 constructs showed the punctate fluorescence expected for lipid droplets. Though the elongated forms resembled epimastigotes, DAPI staining showed no anterior re-positioning of kDNA (a hallmark of epimastigote differentiation), indicating if differentiation was initiated, it did not proceed to epimastigote stage.

Introduction of elongated cells into extremely low glucose media (SDM79θ) caused reversion to WT morphology and restored cell division. Re-introduction of SDM79θ-grown cells back into SDM79 recapitulated the elongation and cell division arrest phenotype. These results suggest over-expression of LD-targeting proteins in high glucose media triggers lethal elongation that is reversed by growth in low glucose media and may represent abortive differentiation.

69: A unique GCN5 histone acetyltransferase complex mediates gene transcription in Plasmodium falciparum

Miao, JUN (Pennsylvania State University); *li, xiaolian; Liang, Xiaoying; Sebastian, Aswathy; Cui, Liwang (Pennsylvania State University)*

Gcn5 functions as a key histone acetyltransferase (HAT) in a number of multisubunit complexes in a variety of eukaryotes such yeast, fruitfly and mammals. These complexes act as a general co-activator in gene transcription. To characterize the components and function of Gcn5 complex in Plasmodium falciparum, by affinity purification and protein mass spectrometry, we first identified plasmodium falciparum homologues of the yeast proteins GCN5, Ada2 and two plant homeo domain (PHD)-containing proteins, named PfPHD1 and PfPHD2, were co-purified with this complex. Gel filtration and co-immunoprecipitation revealed that this complex has an apparent molecular mass of 250 kDa and contains PfAda2A, PfGcn5, PfPHD1 and PfPHD2 as stable subunits. Deletion of Bromodomain in PfGcn5 caused abnormal parasite growth and a significant decrease of histone H3 K9 acetylation at early asexual stage. Thus, the PfGCN5 complex represents a novel HAT complex, which is distinct from previously identified Gcn5/PCAF-containing complexes from yeast and mammalian cells.

70: Epigenetic Mechanisms and Histone Modifications in Giardia lamblia Antigenic Variation

Orozco, Daniel (University of Louisiana at Monroe); *Garlapati, Srinivas (University of Louisiana Monroe)*

Giardia lamblia is a binucleated, flagellated protozoan parasite responsible for the gastrointestinal disease giardiasis. G. lamblia trophozoites undergo surface antigenic variation and express one variant-specific surface protein (VSP) on their surface. The Giardia lamblia genome contains an estimated 200 different VSP genes with only one VSP being expressed at a time. Recent studies have shown that an RNAi mechanism is involved in down regulating all VSP mRNAs except for one that is expressed on the trophozoite's surface. It has been shown that all VSP transcripts have an antisense RNA strand that binds and degrades the VSP transcripts except for the one to be expressed. These studies demonstrate why only one VSP is able to be expressed while the others are not, but how VSP switching occurs remains unclear. This study is investigating epigenetic mechanisms involved in antigenic variation. G. lamblia trophozoites were transfected with endogenously tagged vsp and screened for VSP expression after being treated with histone deacetylase inhibitors (HDACi). Trophozoites were treated with 2µM concentrations of Splitomycin, Trichostatin A (TSA), Apicidin, M344 and Sodium 4-phenylbutuyrate to determine if the HDACi would inhibit cell growth. Growth inhibition was only observed at 2 µM concentrations with Apicidin and TSA. RT-PCR assays indicated endogenously tagged VSP 1267 and VSP 9B10 expression was upregulated after treating trophozoites with 2 µM concentrations of HDAC inhibitors for 24 hours compared to control trophozoites. In order to investigate the role of histone modifications in antigenic variation, two of the six putative histone deacetylase enzymes (HDAC 1 and NAD-dependent histone deacetylase Sir2) will be endogenously tagged and knocked down using viral-mediated hammerhead ribozyme to observe the effects on VSP expression.

71: Using Riboswitch to Control Internal Ribosome Entry Site Mediated Translation Initiation in Giardiavirus

McMahan, Timothy S. (University of Louisiana at Monroe)

Riboswitches are sequences of RNA capable of binding a ligand, inducing a conformational change that affects translation of a gene. The discovery of riboswitches has aided molecular biology research as a means of controlled expression of a gene of interest. This project aims to engineer Giardia lamblia virus (GLV) with a riboswitch in the internal ribosome entry site (IRES) that will allow control over viral gene expression in the host Giardia lamblia. Several small molecule-binding riboswitches were chosen as possible candidates for successful GLV gene regulation including tetracycline, neomycin, and theophylline riboswitches. The viral construct pC631rluc contains the reporter gene renilla luciferase (rluc) driven by viral IRES. Using site-directed mutagenesis we will replace a key stem-loop structure in the IRES element with one of the selected riboswitches. The rationale is that a riboswitch assumes a stable secondary structure when it binds to its cognate ligand and it will then facilitate binding of a ribosome to the IRES element to initiate translation.

Dicistronic viral constructs have also been synthesized containing two IRES elements, one promoting a puromycin resistance gene (pac) and the other promoting the rluc gene. The addition of pac will allow for a relatively stable cell line through a selection process for puromycin resistant cells. Another possible use of the dicistronic system would be to have each IRES control a unique reporter gene and engineer the riboswitch into only one of the IRES elements, leaving the other reporter gene as an internal control.

72: Developing a scalable functional genetics pipeline for the malaria parasite, Plasmodium falciparum

Esherick, Lisl Y. (Massachusetts Institute of Technology)*; McGuffie, Bryan A. (Broad Institute); Nasamu, Sebastian (WUSTL); Dey, Vishakha (IIT Bombay); Dvorin, Jeffrey D. (Boston Children's Hospital); Niles, Jacquin C. (Massachusetts Institute of Technology)*

Given the continuing emergence of Plasmodium falciparum parasites resistant to frontline antimalarials, there is an urgent need to develop new antimalarial drugs, especially those with novel mechanisms of action. Systematic identification and characterization of essential P. falciparum genes can guide drug discovery efforts towards targeting novel genes and/or pathways to reduce the risk of new therapeutics being rapidly compromised due to cross-resistance. However, the limited genetic tractability of P. falciparum has previously posed significant research challenges and, as a result, much of its genome remains functionally uncharacterized. The development of CRISPR-based tools for genetic manipulation has increased the efficiency of gene disruption and tagging in P. falciparum. Using these tools, we are developing a scalable CRISPR-based genetic platform based on the TetR-DOZI system for conditional translational knockdown. By using a conditional knockdown system, we are able to generate lines targeting essential genes by maintaining parasites under permissive conditions. In creating this platform, we are developing methods for parallel assembly of donor vectors for homology-directed repair, as well as pooled assays in which the growth of individual parasite lines can be deconvoluted by next generation sequencing. In assays comparing parasite growth under knockdown and permissive conditions, we find strong concordance between results obtained from small-scale pools and individually assayed parasite lines. By scaling up this resource, we will create arrayed and pooled libraries of transgenic parasites that should serve as a useful starting point for drug-discovery efforts and promote detailed study of essential gene functions in P. falciparum.

73: Novel Method to Study Multigene Families in Plasmodium falciparum

Omelianczyk, Radoslaw (Nanyang Technological University)*; loh, han ping (NTU school of biological sciences); Preiser, Peter (Nanyang Technological University)*

Plasmodium falciparum extensively modifies the red blood cell after invasion. Among the exported proteins are several multigene families which are incorporated into the host cell membrane and exposed to the extracellular space. These proteins are involved in host immune evasion, binding to uninfected red blood cells and interaction with host endothelial cells. Each multigene family consists of dozens of members which are similar among each other, yet differ in their exact amino acid sequence. P.falciparum expresses 3 main families, namely PfEMP1, RIFIN and STEVOR proteins. Studies of these multigene families in vivo have been notoriously difficult. Each parasite expresses only a limited subset of its repertoire (1-3) of a given family and switches expression regularly during laboratory culture. Even though it is possible to select for certain phenotypes (e.g. binding to specific endothelial receptors), the procedure is restricted to proteins with a function that can be selected for and needs to be repeated periodically. Alternatively, specific members can be episomally overexpressed. Overexpression fails to take into account the complementary epigenetic mechanisms that govern the mutual exclusive expression within the multigene families. In this work we describe a novel method to force expression of any member of a multigene family under its endogenous promoter. Since the protein is expressed from its native locus the expression pattern of the remaining multigene members changes accordingly and commonly results in downregulation of the other genes. This method not only allows us to study the function of any multigene protein of choice, but also enables us to investigate the epigenetic control mechanisms that govern the mutual exclusive expression. Finally, we are able to use this method not only for the before mentioned P.falciparum multigenes but can be applied to other Plasmodium spp. as well.

74: Engineering a self-targeting entry inhibitor for vectored malaria prophylaxis

Srinivasan, Prakash (Johns Hopkins Malaria Research Institute, Dept. Molecular Microbiology and Immunology)*; Xiao, Shuhao (Johns Hopkins Malaria Research Institute); Pandey, Rajeev (Johns Hopkins Malaria Research Institute); Bell, Cameron (Johns Hopkins Malaria Research Institute); Verma, Garima (Johns Hopkins Malaria Research Institute); Ketner, Gary (Johns Hopkins Malaria Research Institute, Dept. Molecular Microbiology and Immunology)*

A critical step in Plasmodium falciparum (Pf) host cell invasion is the formation of a tight junction between the parasite and the host cell, which provides a firm anchor to facilitate parasite entry. This step requires interaction between the micronemal protein AMA1 and RON2, a component of the parasite secreted RON receptor complex. This interaction is also required for efficient Pf sporozoite invasion of hepatocytes. This is a unique example in host-pathogen interactions where both ligand and receptor are provided by the parasite for successful host cell entry. We recently showed that a 49 amino acid region of RON2 (termed RON2L) is sufficient to bind AMA1 and block invasion, thereby acting as a potent entry inhibitor. RON2L and its binding pocket in AMA1 is conserved within each Plasmodium species and points to an evolutionarily preserved “lock-and-key” mechanism designed to prevent changes. We reasoned that targeting this interaction can be an effective antimalarial strategy as it would make it harder for the parasites to develop resistance. To do this, we are developing a novel gene therapy approach using adeno-associated virus (AAV) to endogenously express the entry inhibitor RON2L masked as a human Ig fusion protein (RON2L-Ig). We show that the entry inhibitor expressed by AAV transduction is produced as a correctly folded, stable fusion protein and potently neutralized P. falciparum merozoites in vitro. We are using a in vivo rodent malaria model and a P. falciparum humanized mouse model to evaluate protection against infection and disease. Our data indicate that self-targeting this critical invasion pathway used by both the transmitting and disease causing forms of Plasmodium could provide an effective prophylaxis tool that can augment current malaria elimination efforts.

Poster Session B

Tuesday, September 11, 2018

7:00 pm – 9:00 pm

Poster Numbers 75 - 149

For Abstract see Session V, TT1
75: DOC2 domain proteins in Toxoplasma gondii Ca2+-dependent secretion
Tagoe, Daniel A.; *Coleman, Bradley; Stoneburner, Emily; Drozda, Allison; Coppens, Isabelle; Gubbels, Marc-Jan*

For Abstract see Session V, TT2
76: Blood donor variability as a modulatory factor in Plasmodium falciparum invasion phenotyping assays
Thiam, Laty G*.; Aniweh, Yaw; kusi, Kwadwo A.; Niang, Makhtar; Gwira, Theresa M.; Awandare, Gordon A.*

For Abstract see Session V, TT3
77: A novel role for EXP2 in invasion of Plasmodium sporozoites
Mello-Vieira, Joao*; de Koning-Ward, Tania; Mota, Maria; Zuzarte-Luís, Vanessa*

For Abstract see Session V, TT4
78: Impact of malaria-protective glycophorin polymorphism on Plasmodium falciparum invasion
Kariuki, Silvia N*.; Marin-Menendez, Alejandro; Leffler, Ellen; Band, Gavin; Rockett, Kirk; Macharia, Alex; Makale, Johnstone; Nyamu, Wilfred; Ndung'u, Francis; Kwiatkowski, Dominic; Williams, Thomas; Rayner, Julian*

For Abstract see Session V, TT5
79: Comparative assessment of PbSLTRiP (Sporozoite and Liver stage expressed Tryptophan Rich Protein) peptides as vaccine candidates against Plasmodium berghei in mice.
Quadiri, Afshana*; Singh, Agam Prasad*

For Abstract see Session VI, TT1
80: TgMyoF is an organizer of the endosome-like compartment in Toxoplasma gondii
Carmeille, Romain*; Heaslip, Aoife*

For Abstract see Session VII, TT1

81: Cathepsin L (TBCATL) Processing And Post-Golgi Sorting To The Lysosome In African Trypanosomes

Koeller, Carolina M*.; Bangs, James D.*

For Abstract see Session VII, TT2

82: Characterization of vesicular systems of artemisinin resistance suggest their roles in trafficking and virulence, dependent and independent of Kelch13

Suresh, Niraja*; Khair, Maisha; Coppens, Isabelle; Bhattacharjee, Souvik; Mbengue, Alassane; Ghorbal, Mehdi; Haldar, Kasturi*

83: Rapid, iterative and scalable genome editing in zoonotic malaria parasite Plasmodium knowlesi

***Mohring, Franziska (London School of Hygiene and Tropical Medicine)**; Hart, Melissa N. (London School of Hygiene and Tropical Medicine); Rawlinson, Thomas (Jenner Institute Oxford); Henrici, Ryan (London School of Hygiene and Tropical Medicine); Patel, Avnish (London School of Hygiene and Tropical Medicine); Baker, David (London School of Hygiene and Tropical Medicine); Sutherland, Colin (London School of Hygiene and Tropical Medicine); Draper, Simon (Jenner Institute Oxford); Moon, Robert W (London School of Hygiene and Tropical Medicine)*

Malaria remains a serious health burden globally, with over 216 million cases annually. Whilst eradication efforts are making progress in some areas, dealing with relapsing infections and emerging threats like antimalarial resistance and zoonotic malaria infections will require new tools and approaches. Plasmodium vivax still lacks a long-term in vitro culture system, therefore vaccine development has had to rely on in vitro binding assays with recombinant protein, ex vivo studies, primate infections and controlled human malaria infections. Thus, scalable parasitological assays to support extensive testing of antisera and antigens, prior to escalation to in vivo work, is desperately needed. Adaptation of the clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 system to the most severe malaria parasite P. falciparum revolutionised the field, however P. falciparum is genetically most divergent to all other human malaria parasites.We established CRISPR-Cas9 genome editing in P. knowlesi to enable rapid, iterative and scalable targeted modifications of the parasite genome. Using an optimised scalable PCR-based approach for generating targeting constructs we have defined critical parameters affecting success rates and applied the technique to introduce genetic tags to a variety of proteins with distinct cellular localisations. Furthermore, we replaced the full-length P. knowlesi Duffy binding protein PkDBPa with its P. vivax DBP orthologue, an adhesion protein essential for human red blood cell invasion and leading vaccine candidate. Finally, we iteratively deleted two other P. knowlesi paralogues, to generate a transgenic P. knowlesi line that can be used for optimisation of vaccines against P. vivax.

84: A patatin-like phospholipase is involved in gametocytogenesis of the human malaria parasite Plasmodium falciparum

Flammersfeld, Ansgar (Division of Cellular and Applied Infection Biology, Institute of Zoology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany)*; Lang, Christina (Division of Enteropathogenic Bacteria and Legionella, Robert Koch- Institute, Burgstraße 37, 38855 Wernigerode, Germany); Flieger, Antje (Division of Enteropathogenic Bacteria and Legionella, Robert Koch- Institute, Burgstraße 37, 38855 Wernigerode, Germany); Sollelis, Lauriane (Wellcome Center for Molecular Parasitology, University of Glasgow, 120 University Place, Glasgow, Scotland); Marti, Matthias (Wellcome Center for Molecular Parasitology, University of Glasgow, 120 University Place, Glasgow, Scotland); Ngwa, Che Julius (Division of Cellular and Applied Infection Biology, Institute of Zoology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany); Pradel, Gabriele (Division of Cellular and Applied Infection Biology, Institute of Zoology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany)*

The genome of Plasmodium falciparum encodes four proteins with a patatin-like phospholipase domain (PNPLAs). Such domains have a Ser-Asp catalytic dyad and display lipase and transacylase properties. PNPLAs are found both in prokaryotic and eukaryotic organisms and have major roles in lipid and energy homeostasis. In P. falciparum, the four PNPLAs are thought to mediate the conversion of phosphatidylcholine into lysophosphatidylcholine (LysoPC) as well as its further processing. Noteworthy, LysoPC was recently identified as an environmental sensor repressing sexual commitment in P. falciparum, i.e., LysoPC restriction triggers the switch from the clinically relevant asexual blood stage parasite to the transmissive gametocyte stages. We here investigated the role of one of the four plasmodial PNPLAs, a putative PLA2 termed PNPLA1, during blood stage replication and gametocyte development. Transcript and protein expression analysis revealed that PNPLA1 is expressed in both parasite stages and localizes to the cytosol.

PNPLA1-deficient parasite lines were generated using conventional gene disruption as well as conditional glmS-based gene-knockdown approaches. Phenotype analyses demonstrated that depletion of PNPLA1 only had a minor effect on erythrocytic replication and gametogenesis, and the asexual blood stages and gametocytes showed normal morphology compared to the wildtype. However, PNPLA1-deficient parasites had reduced gametocyte numbers, suggesting a role in either sexual commitment or early gametocyte development. Experiments are ongoing to define the precise role of this enzyme in these processes.

85: Analysis of Plasmodium berghei infection and occupancy of Anopheles stephensi salivary glands

Wells, Michael B. (Johns Hopkins University)*; Villamor, Jordan (Johns Hopkins University School of Medicine); Andrew, Deborah (Johns Hopkins University School of Medicine)*

Mosquito-borne diseases, such as malaria, continue to cause hundreds of thousands of deaths each year. For malaria-causing parasites (Plasmodium spp.) to be transmitted to humans, sporozoites enter the salivary glands (SGs), traverse secretory cells and accumulate in the central lumen. Infective sporozoites travel with saliva through the narrow salivary duct to leave the mosquito. Detailed analyses of SG invasion by Plasmodium parasites have been limited to electron microscopy studies, the most detailed of which focused on SG invasion of Aedes mosquitoes by avian malaria parasites. Here, we used fluorescence confocal microscopy to investigate Anopheles stephensi SG invasion by Plasmodium berghei, allowing examination of hundreds of mosquitoes over a wide range of infection intensities. We found that many aspects of invasion are conserved across mosquito and parasite species, including distal lateral lobe-focused invasion, cell stress or death, SG basement membrane repair, parasite bundling, and the presence of transient parasitophorous vacuoles. We made novel observations about SG infection involving sites of subcellular SG localization of two parasite proteins (CSP and TRAP), reduced saliva protein levels, low sporozoite duct occupancy, sporozoite emergence from parasitophorous vacuoles, parasite maturation in the SG, direct to lumen traversal, parasite orientation within bundles, pooled parasite bundle-associated apical secretions, and SG architecture effects on cell traversal by sporozoites. Through extensive quantification, we find consistency between the two parasite lines we tested as well as before and after a second, noninfective blood meal is given. Altogether, this work identifies conserved features of SG invasion, establishes methods for evaluating
SG-based transmission interventions, indicates that parasite invasion influences SG structure and secretion, and suggests that SG architecture is a key determinant of parasite availability for transmission.

86: Plasmodium falciparum Phosphatidylinositol 3'-Kinase is a Novel Target for the Development of Next-Generation Artemisinin Combination Therapies

Iyengar, Kalpana (Departments of Chemistry, and of Biochemistry and Cellular and Molecular Biology, Georgetown University, 37th and O St. NW, Washington, DC.); *Siriwardana, Amila (Departments of Chemistry, and of Biochemistry and Cellular and Molecular Biology, Georgetown University, 37th and O St. NW, Washington, DC.); Hassett, Matthew (Departments of Chemistry, and of Biochemistry and Cellular and Molecular Biology, Georgetown University, 37th and O St. NW, Washington, DC.); Eastman, Richard (Preclinical Innovation, National Center for Advancing Translational Sciences, NIH); Thomas, Craig (Preclinical Innovation, National Center for Advancing Translational Sciences, NIH); Roepe, Paul (Departments of Chemistry, and of Biochemistry and Cellular and Molecular Biology, Georgetown University, 37th and O St. NW, Washington, DC.)*

Several studies have shown that Plasmodium falciparum parasites possess the machinery necessary for autophagy and that this pathway is triggered in response to starvation conditions or to LD50 (lethal) concentrations of drugs such as artemether or chloroquine. Vps34 (a class III phosphatidylinositol 3'kinase) is a key regulatory enzyme in the autophagy cascade in eukaryotic cells and the P. falciparum genome encodes a unique Vps34. Previous studies from our laboratory have shown that PfVps34 is indeed a "class III" PI3'K- or Vps34 enzyme. We have also shown that several known PI3'K inhibitors are extremely potent antimalarial compounds at both IC50 (growth inhibitory) and LD50 (lethal) concentrations. Furthermore, PI3'K inhibitor : artemisinin-drug combinations are synergistic. Due to the increasing occurrence of resistance to current artemisinin (ART) combination therapies (ACTs), new ACTs are desperately needed. Here, we show using immunofluorescence assays (IFAs) that PI3'K inhibitors inhibit the autophagy pathway and that the extent of inhibition is correlated with both the in vitro lethal dose of the respective compound and the activity of the compound against purified PfVps34 enzyme. Additionally, attempts to select parasites resistant to NVP-BGT226 (an extremely potent PI3'K inhibitor) using multiple methods have not been successful, likely due to the fact that PfVps34 appears to be essential and is present in only one copy in the malaria genome. These data indicate that PfVps34 is a valuable new drug target and that ART : anti-PfVps34 drug combinations are important new candidate ACTs. Also, increased levels of PI3P (phosphatidylinositol 3'-phosphate – the sole product of PfVps34) have been implicated in the decreased sensitivity of P. falciparum parasites to ART - based drugs. Therefore, we further investigated this target in Cambodian isolates harboring ART delayed clearance phenotype (DCP) conferring kelch-13 (K13) mutations. We find that K13-mutant parasites exhibit a muted autophagy response relative to K13 wild-type parasites that is efficiently inhibited by ART : anti PfVps34 drug combinations. Our data implicate PfVps34 as a novel, imminently druggable target in ART-DCP P. falciparum parasites.

87: Plasmodium sERAD is essential for apicoplast maintenance

***Rajaram, Krithika (Johns Hopkins Malaria Research Institute)**; Prigge, Sean*

Endosymbiotic events in the evolutionary history of Plasmodium falciparum led to the retention of a four-membraned plastid called the apicoplast. The production of isoprenoid precursors (IP) appears to be the only essential function of the apicoplast in blood-stage P. falciparum. Thus, parasites treated with apicoplast-targeting drugs can still survive in the presence of an IP source. Our lab previously created a parasite line (PfMev) that cytosolically expresses enzymes from an alternative isoprenoid pathway to convert supplied mevalonate to IP. In this background, we can assess the essentiality of apicoplast proteins in organellar maintenance and/or parasite survival. We employed the PfMev line to characterize a symbiont-specific endoplasmic reticulum-associated protein degradation (sERAD) translocon. The vast majority of apicoplast proteins are nuclear-encoded and are imported into the plastid post-translationally. In related chromalveolates, sERAD localizes to the second outermost plastidial membrane and involves the participation of ubiquitinating enzymes to mobilize substrates across the membrane. To determine if sERAD has a similar function in P. falciparum, we utilized CRISPR/Cas9 to disrupt three putative sERAD genes in PfMev parasites, namely DER1-1, UBC-1 (ubiquitin conjugating enzyme E2) and PUBL (plastidial ubiquitin-like protein). All three knockout parasite lines were dependent on mevalonate for survival and no longer possessed intact apicoplasts. PUBL is unique to Plasmodium and contains a ubiquitin-like fold and an unknown C-terminal domain. To further characterize this protein, we placed PUBL under the regulatable control of the TetR-DOZI system. Upon PUBL depletion, parasites were impaired in growth and a large proportion of parasites contained disrupted apicoplasts. Our results show that sERAD in P. falciparum is required for apicoplast integrity. Ongoing studies will determine if PUBL is involved in protein import and whether it interacts with protein substrates or other components of sERAD.

88: The news about ISC system in the mitosomes of Giardia intestinalis

Motyckova, Alzbeta (Department of Parasitology, BIOCEV, Faculty of Science, Charles University in Prague, Czech Republic)*; Stairs, Courtney (Department of Cell and Molecular Biology, Uppsala University, Sweden); Najdrova, Vladimira (Department of Parasitology, BIOCEV, Faculty of Science, Charles University in Prague, Czech Republic); Voleman, Lubos (Department of Parasitology, BIOCEV, Faculty of Science, Charles University in Prague, Czech Republic); Dolezal, Pavel (Department of Parasitology, BIOCEV, Faculty of Science, Charles University in Prague, Czech Republic)*

Mitosomes of Giardia intestinalis are the most reduced mitochondria found to date. They do not have any DNA and their proteome is extensively reduced - just a few tens of proteins are identified as mitosomal. The only known metabolic pathway is the synthesis of iron-sulphur clusters (ISC system).There are many unknowns in mitosome biology mainly concerning its biogenesis, the transport of biomolecules across mitosomal membranes and the actual role of mitosomal ISC pathway for the function of other cellular compartments. In this project, we tackle these question by two biochemical approaches. (i) we attempt to establish affinity purification of the whole mitosomes from cell lysate. The technique is based on specific biotinylation of the outer mitosomal membrane proteins. Now we are testing the most suitable protein candidates. (ii) we also purify individual component of ISC pathway to identify missing functional components and the long sought substrate(s) of the pathway. Recently, we identified G. intestinalis BolA homologue, which is normally present in ISC pathway of only aerobic organisms. We localized BolA into mitosomal matrix, identified its interacting partners, which all belong to ISC pathway. We were able to show that BolA interact strongly with glutaredoxin 5, as known for aerobic mitochondria of yeast and we also tested functional residue in this interaction. This discovery raises a new exciting question about yet unknown putative substrate of these mitosomal ISC components or its relation to the iron-sulfur cluster assembly in other cellular compartments.

89: Visualizing and identifying secreted effectors from Toxoplasma gondii bradyzoites

Mayoral, Joshua (Albert Einstein College of Medicine); *Tu, Vincent (Albert Einstein College of Medicine); Tomita, Tadakimi (Albert Einstein College of Medicine); Ma, Yanfen (Albert Einstein College of Medicine); Weiss, Louis (Albert Einstein College of Medicine)*

Toxoplasma gondii is an obligate intracellular parasite of the phylum Apicomplexa. Acute infection is characterized by tachyzoites, fast-replicating life stages that disseminate throughout the warm-blooded host. Chronic (latent) infection is characterized by bradyzoites, slowly-replicating life stages which encyst in muscle and neural tissue. Bradyzoite biology is poorly understood; how bradyzoites manipulate host cell function and persist within host cells indefinitely is unknown. Previous studies have demonstrated the secretion of various protein effectors by tachyzoites into their host cells across the parasitophorous vacuole, such as GRA16, GRA24, GRA28, and TgIST. Most of these effectors have been shown to be transported to the host cell nucleus and interact with host cell proteins, thereby affecting host cell signaling and transcription. Whether these, or other, unidentified proteins, are exported by bradyzoites has not been previously explored. In this project, we aim to determine the localization of GRA16, GRA24, GRA28, and TgIST after bradyzoite differentiation in vitro via epitope tagging and in vivo in the mouse brain using an optical clearing approach. To identify novel exported parasite proteins, proximity based biotinylation approaches (BirA) will be utilized by fusing enzymatic tags to MYR1, a protein implicated in parasite protein delivery into the host cell. Discovering novel exported bradyzoite proteins which enter the host cell will lead to further studies toward understanding how bradyzoites alter host cell function and maintain persistency within their host cell. Such findings would challenge a paradigm in the field of Toxoplasma research, where the bradyzoite has been classically viewed as an inert stage of the parasite. [Supported by NIH AI134753 (LMW)].

90: Identifying the mechanism of action of Tartrolon E, a broad spectrum anti-apicomplexan compound

Bowden, Gregory (Washington State University)*; Driskell, Iwona (Washington State University); Nepveux, Felix (Tufts Medical Center); Lin, Zhenjian (University of Utah); Schmidt, Eric (University of Utah); Schafer, Deborah (University of Arizona); Riggs, Michael (University of Arizona); O'Connor, Roberta (Washington State University)*

The apicomplexan parasite Cryptosporidium is the cause of the severe diarrheal disease cryptosporidiosis. Cryptosporidium is the most common parasitic waterborne infection in the United States; young children and immunocompromised individuals are particularly susceptible to morbidity and mortality due to infection. Nitazoxinide, the only drug approved for treatment of cryptosporidiosis, is ineffective in immunocompromised patients, thus new therapeutics are a high priority. We have identified Tartrolon E (TrtE), a secondary metabolite derived from shipworm symbiotic bacteria with broad-spectrum anti-apicomplexan parasite activity. TrtE inhibits Cryptosporidium at nM concentrations in vitro and is highly effective inhibiting infection in vivo. As Cryptosporidium is difficult to manipulate, we elected to investigate the mechanism of action of TrtE using the model apicomplexan parasite Toxoplasma gondii. Since repeated attempts to generate TrtE-resistant T. gondii mutants were unsuccessful, we investigated changes in the transcriptome of TrtE treated T. gondii. RNA-sequencing data revealed that the conserved gene TGME49_272370 is significantly upregulated within four hours of treatment. This result was confirmed by RT-qPCR, where it was further determined that TGME49_272370 expression during treatment with TrtE was dose-dependent. These data suggested a role for TGME49_272370 in the parasite's response to TrtE treatment. The function of the protein product of TGME49_272370 is unknown. However, the protein product is predicted to have multiple transmembrane domains and potential phosphorylation sites. To examine the effect of TGME49_272370 on parasite susceptibility to TrtE, we will produce TGME49_272370 deletion mutants using CRISPR/Cas9. In uncovering the mechanism by which TrtE inhibits T. gondii parasites, we may identify a shared pathway critical to apicomplexan parasite survival and advance the search for new treatments for cryptosporidiosis.

91: Polyunsaturated fatty acids promote Plasmodium falciparum gametocytogenesis

Tanaka, Takeshi (Kagawa University); *Tokuoka, Suzumi (The University of Tokyo); Nakatani, Daichi (Obihiro University of Agriculture and Veterinary Medicine); Hamano, Fumie (National Center for Global Health and Medicine); Kawazu, Shin-ichiro (Obihiro University of Agriculture and Veterinary Medicine); Wellems, Thomas (NIAID/NIH); Kita, Kiyoshi (Nagasaki University); Shimizu, Takao (National Center for Global Health and Medicine); Tokumasu, Fuyuki (The University of Tokyo)*

Gametocytogenesis of Plasmodium falciparum undergoes in a small fraction of intraerythrocytic stage parasite as a sexual differentiation. Intracellular factors involved in the gametocytogenesis have been suggested, but critical extracellular factors for stimulating parasites to emerge from the asexual stages remain unresolved. In in vitro culture, human serum as an additive for culturing gametocytes instead of a commercially available serum substitute produces better gametocytemia, suggesting that serum components missing in commercial product help to trigger gametocytogenesis. Microscopic analyses revealed that gametocytes have more lipids inside cells, suggesting that lipid components could be key factors to support gametocyte development. In this study, we focused on the contributions of lipids to efficient gametocytogenesis and found that polyunsaturated fatty acids (PUFA) boost the production of gametocytes. Lipidomics study for the phospholipid and fatty acid profiles revealed that phosphatidylcholine (PC) and phosphatidylethanolamine (PE) with PUFA were more abundant in gametocytes than in the asexual stages, and the entire profile became more resembled to the serum fatty acid profile. This profile similarity also occurred with neutral lipids, suggesting that extracellular lipids are important to gametocyte lipid profiles. To test this hypothesis, we supplemented extra phospholipids by adding PUFA to the Albumax-containing culture media. Gametocytemia with arachidonic acid and docosahexanoic acid was increased to levels comparable to those achieved with human serum. This effect was not observed with a saturated palmitic acid. Our results suggest that the balance of extracellular lipids with PUFA is important to the promotion of gametocytogenesis.

92: PfEMP1 proteins binding non-immune IgM are common among Plasmodium falciparum parasites

Quintana, Maria del Pilar (Centre for Medical Parasitology, University of Copenhagen); *Ditlev, Sisse (Centre for Medical Parasitology, University of Copenhagen); Ecklu-Mensah, Gertrude (Centre for Medical Parasitology, University of Copenhagen); Hviid, Lars (Centre for Medical Parasitology, University of Copenhagen); Lopez-Perez, Mary (Centre for Medical Parasitology, University of Copenhagen)*

Plasmodium falciparum virulence is related to the capacity of the infected erythrocytes (IEs) to adhere to host receptors located on vascular endothelial cells or on uninfected erythrocytes. IE adhesion is mostly mediated by the family of parasite-derived surface antigens P. falciparum erythrocyte membrane protein-1 (PfEMP1). Efficient binding of specific PfEMP1 variants to their host receptors is augmented by human serum factors, such as non-immune IgM and a2-macroglobulin (a2M).Given the apparent importance of the binding of these serum factors to the IEs via PfEMP1, we aimed to identify all IgM-binding PfEMP1 variants in three different P. falciparum clones (3D7, HB3 and IT4). We used parasites transfected with the pVBH plasmid, which have a heterogeneous expression of PfEMP1 variants. IEs labelled with non-immune human IgM were single-cell sorted by FACS and the transcription of PfEMP1-encoding var genes by descendants of individual IgM+ IEs identified by qPCR. Since the IgM- and a2M-binding phenotypes have been reported to be correlated, we tested if the IgM-binding IEs were also able to bind a2M. The binding ability of some of the identified PfEMP1 candidates was validated by ELISA, using recombinant proteins representing single and multiple PfEMP1 domains.We report here that several PfEMP1 variants within a single parasite genome can bind non-immune IgM. The newly identified candidates include variants containing DBLe and DBL? domains, which have been previously reported to bind non-immune IgM in other PfEMP1 variants. However, we also identified IgM-binding PfEMP1 variants without these domain types, indicating that binding of non-immune IgM could be more frequent than previously assumed, perhaps suggesting functional significance.

93: Identification of Toxoplasma gondii genes that determine fitness in interferon gamma-stimulated rodent macrophages

Wang, Yifan (Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA)*; Sangaré, Lamba Omar (Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA); Krishnamurthy, Shruthi (Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA); Paredes-Santos, Tatiana (Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA); Sidik, Saima (Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA); Lourido, Sebastian (Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA); Saeij, Jeroen (Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA)*

Toxoplasma gondii is an obligate intracellular parasite that infects almost all warm-blooded animals. Among its different hosts there are natural differences in susceptibility to the parasite. Mice are susceptible to infection whereas rats are relatively resistant. Macrophages play an essential role in the early immune response against Toxoplasma and are the major cell type preferentially infected in vivo. Interferon gamma (IFNγ) activates a variety of anti-Toxoplasma activities in macrophages. However, Toxoplasma can block IFNγ-induced parasite restriction mechanisms using effectors secreted from its unique secretory organelles. Thus, knowing the parasite effectors that specifically counteract the IFNγ response in macrophages from different hosts will help us better understand the molecular basis of host susceptibility. We used a genome wide CRISRP/Cas9 loss-of-function screen to identify Toxoplasma genes that determine fitness in IFNγ-stimulated macrophages from mice and rats. We identified ~20 previously uncharacterized fitness-conferring genes in IFNγ-stimulated rodent macrophages, 3 of which confer fitness in both mouse and rat IFNγ-stimulated macrophages whereas 18 genes confer fitness only in mouse or rat. This suggests that Toxoplasma has developed different strategies to establish infection in different hosts. We have generated individual knockout parasites for a number of these genes. Growth competition with wild-type parasites showed that parasites lacking GRA22, one of the common hits from both screens, have a fitness defect in both rat and mouse IFNγ-stimulated macrophages. We are in the process of determining the exact mechanism by which these Toxoplasma genes determine fitness in IFNγ-stimulated rodent macrophages.

94: An essential Flagellum Pocket Collar protein of Trypanosoma brucei that localises to the centrosome when expressed in mammalian cells

Reix, Christine E (University of Bordeaux); *Cayrel, Anne (University of Bordeaux); Florimond, Celia (University of Buffalo); Landrein, Nicolas (University of Bordeaux); Dacheux, Denis (University of Bordeaux); Morriswood, Brooke (University of Wurzburg); Robinson, Derrick (University of Bordeaux)*

Trypanosomes are ubiquitous parasites causing deadly human and veterinary diseases. No vaccines and only limited therapeutic drugs are available; hence new drug targets are crucial (1)?. These evolutionary ancient eukaryotes are interesting models to study ciliopathies and spermatozoa flagellum defects (2,3). Studies of a trypanosome essential organelle, the flagellar pocket (FP) (4)?, identified two essential proteins: BILBO1 of the FP collar (FPC) (5)? and MORN1 of the Hook complex (6)?. FPC6 is a kinetoplastid specific, cytoskeletal protein. When endogenously tagged in Trypanosoma brucei FPC6 is expressed throughout the cell cycle, partially co-localises with BILBO1 and fully with MORN1, displaying a hook-shape and curving around the flagellum. Surprisingly, FPC6 expression in mammalian U2-OS cells shows localisation with the centrosome and in RPE-1 cells with the cilia basal body. FPC6 depletion, using RNAi (7)?, in cultured procyclic forms (PCF) of T. brucei shows knock-down followed by revertants with up-regulated protein levels; no growth defect is observed. FPC6 depletion in bloodstream forms (BSF) reveals a severe growth defect, defective kinetoplast segregation and cell death.These data confirm the permanent presence of FPC6 protein within the hook complex at the FPC, and the essentially of FPC6 in the BSF (8)?, indicating the importance of FPC6 for the pathogen. Expression in mammalian cells infers that FPC6 has a predilection for axonemal or MTOC (microtubule organising centre) related structures. Future studies will involve expressing optimised FPC6 truncations in trypanosomes to characterise domain targeting and the ability to rescue RNAi knockdown; expression in a heterologous system to identify BILBO1 and MORN1 binding and polymer-forming properties; determination of FPC6 function in vivo, using endocytotic assays.

95: Heme Activation of Artemisinin Antimalarial Drugs

Heller, Laura (Georgetown University); *Roepe, Paul (Georgetown University)*

The current standard of care for Plasmodium falciparum malaria is one of several currently available Artemisinin (ART) combination therapies.
Artemisinin, derived from the Chinese herb Artemisia annua, contains a highly reactive 1,2,4 trioxane ring. In its reduced form, free ferriprotoporphyrin IX heme is known to catalyze endoperoxide cleavage for artemisinin drugs. The activated drug likely proceeds from an oxy radical form to a carbon centered radical form that is then capable of alkylating a variety of drug targets within malarial parasites. It has been proposed for some time that heme liberated upon hemoglobin catabolism is one such target, a hypothesis that is supported by direct evidence for heme-ART adducts in artemisinin-treated mice infected with Plasmodium vinckei petteri [Meunier 2002]. Using optimized extraction procedures, mass spectrometry and UV-Visible spectroscopy, we have quantified the abundance of heme and hemozoin at various stages of the P. falciparum life cycle for artemisinin-sensitive and delayed clearance phenotype parasites. Additionally, artemisinin drug - heme adducts from bolus dosed parasites have been identified. These data are important for defining the molecular pharmacology of artemisinin antimalarial drugs.

96: Low mutation rate during Eimeria maxima precocity selection

Hu, Dandan (College of Veterinary Medicine, China Agricultural University)*; Wang, Chaoyue (College of Veterinary Medicine, China Agricultural University); Liu, Xianyong (College of Veterinary Medicine, China Agricultural University); Suo, Xun (College of Veterinary Medicine, China Agricultural University)*

Economically important Apicomplexa parasites Eimeria species undergo both sexual and asexual development in a single host and have small genome size, which make them a good model for studying developmental biology. Here, we use Eimeria maxima to study the genetic variation responsible for changes of developmental stages under directional selection. A high standard 52Mb reference genome of E. maxima Beijing strain (55 contigs, N50=2.26Mb) was assembled using 6.2 Gb PacBio clean data. Then, a precocious line (with greatly reduced prepatent time and fecundity) of the Beijing strain was selected by sequential passage of 22 generations of very early shed oocysts in the host chickens. Genome DNA of selected intermediate generations (G1, G4, G7, G9, G12, G18, G20, G22 and G30) and 4 other E. maxima geographical isolates were subjected to Illumina deep sequencing (88~125X coverage). We found 135458~193970 SNPs between geographical isolates and the Beijing strain, however, only 215 ~ 286 mutations were detected (mostly in non-coding sequences) between generations during precocity selection in our experimental evolution system. Additionally, the transition/transversion rate of the selected intermediate generations (1.295) were also much lower than the geographical isolates (1.352), indicating a limited divergence during precocity selection.

Among SNPs between selected generations, a non-synonymous coding variation in contig_51 (position 812303) was first emerged at generation-18 and was kept in all the subsequent generations. Unexpectedly, this mutation arose contemporaneously with the sharp reduction of prepatent time, suggesting this locus be related to E. maxima precocity. In conclusion, we obtained a high standard E. maxima reference genome and found low mutation rate during precocity selection. The non-synonymous coding variation related to precocity is being validated by genetic engineering. This work was supported by the National Natural Science Foundation of China (31772728).

97: High-level resistance to Plasmodium falciparum cytochrome B inhibitors maps to residues 126 and 268

***Lane, Kristin D. (NIAID/NIH)**; Mu, Jianbing (1Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health); Liu, Jinghua (2Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health); Windle, Sean T. (1Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health); Sun, Peter D. (2Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health); Wellems, Thomas E. (1Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health)*

The mitochondrial electron transport chain (ETC) of Plasmodium falciparum parasites remains an attractive target for antimalarial development. The P. falciparum ETC inhibitor atovaquone (ATQ) inhibits the quinol oxidation (Qo) pocket of the ETC cytochrome B (CytB) enzyme. We recently reported constant drug pressure with two halogenated quinolones, CK-2-68 and RYL-552, unexpectedly selected for PfCytB Qo mutations. These resistant (R) clones were sequentially selected with ATQ, CK-2-68, or RYL-552, and new patterns of differential drug response were obtained. Notably, RYL-552 selection on the CytB Y268S ATQ-R clone produced a mutant with no secondary CytB mutation that exhibited high-level ATQ resistance relative to the ATQ-R parent (3X increase, EC50 = 22 mM). CK-2-68 selections on RYL-552-R lines (A122T or V259L mutants) produced two distinct clones: 6RC-1B12A122T (A122T), and 6RC-1B92Y126C, V259L (Y126C-V259L). 6RC-1B12A122T rapidly lost high-level resistance to CK-2-68. In contrast, 6RC-1B92 displayed long-term high-level resistance to CK-2-68 and antimycin A, a CytB quinone reduction site inhibitor (6.6X, and 309X respectively). We modeled ATQ and CK-2-68 docked into the wildtype CytB and CytB containing residue substitutions: Y268S (ATQ), and Y126C, V259L, or Y126C-V259L (CK-2-68). The estimated inhibitory constants (Ki) were calculated for each model. The Y268S-ATQ model predicted significantly increased Ki of the drug, whereas the Y268S-CK-2-68 model did not, underscoring the importance of ATQ interactions with the 268 tyrosine. Both the Y126C models showed significantly increased CK-2-68 Ki values, suggesting Y126C underpins high-level CK-2-68 resistance. In contrast, there was no significant change to either the ATQ or RYL-552 Ki in either Y126C model, consistent with observed minor drug response changes.

98: Exploring the regulatory role of the transcription factor ApiAP2 in Toxoplasma gondii sexual development

Wang, Chaoyue (College of Veterinary Medicine, China Agricultural University)*; Tang, Xinming (College of Veterinary Medicine, China Agricultural University); Hu, Dandan (College of Veterinary Medicine, China Agricultural University); Wang, Si (College of Veterinary Medicine, China Agricultural University); Liu, Xianyong (College of Veterinary Medicine, China Agricultural University); Suo, Xun (College of Veterinary Medicine, China Agricultural University)*

Toxoplasma gondii is one of the most common parasitic infections of warm-blooded animals and humans. Understanding the mechanisms responsible for controlling development of the asexual stages in intermediate host and the sexual stages in the feline is critical for preventing toxoplasmosis progression and transmission. A family of apicomplexa-specific transcription factors which contains AP2 DNA-binding domains (ApiAP2) have been identified in malaria parasites and some of them controls the sexual conversion. Subsequently, a dozen of the 67 predicted AP2-coding genes were found to be differentially expressed in merozoites or the sporulation process in T. gondii. To reveal the underlying mechanism of sexual development of T. gondii controlled by the TgAP2, we analyzed the expression level of the differently expressed AP2 genes in various life cycle stages and successfully disrupted six ApiAP2 genes using CRISPR/Cas9 technique in the Prugniaud (Pru) strain. We found that endogenous AP2XII-6 (TGME49_249190) fused with 3xHA tag in its C-terminal was undetectable by neither indirect immunofluorescence assay (IFA) nor western blot indicating its low expression in tachyzoites. So, we constructed a TgAP2XII-6 overexpression strain and found that TgAP2XII-6 was mainly located in the nuclear in tachyzoite stage. We further knockout the TgAP2XII-6 gene and found that the proliferation and virulence of the knockout strain is similar with the Pru strain. However, Kunming mice infected with Pru strain produced an average of 172 brain cysts, those infected with ΔAP2XII-6 yield an increased average of 401 brain cysts. These results indicated that AP2XII-6 is a candidate factor controlling cyst formation. Our further study will be focused on analyzing the underlying mechanism and its potential role in sexual development or in the oocyst formation in a cat model.

99: Structure, function and dynamics of minimalist mitochondria of Giardia intestinalis.

Dolezal, Pavel (Charles University); *Pyrihova, Eva (Charles University); Motyckova, Alzbeta (Charles University); Voleman, Lubos (Charles University); Najdrova, Vladimira (Charles University); Wandyszewska, Natalia (Charles University)*

Mitosomes of the intestinal parasite Giardia intestinalis remain one of the simplest mitochondrial organelles known to date. Despite being such tiny organelles, our recent analyses of mitosomal proteome showed that in addition to the newly found components of the extended iron-sulfur cluster pathway, there is a number of Giardia-specific proteins with unknown function, which we currently investigate. Moreover, by following the mitosomes in live using fluorescent tag compatible with the anaerobic environment, we were able to show that their dynamics is strictly controlled by the cell and life–cycle of the parasite. Similarly, the structural organization of the individual mitosomes shows that two mitosomal membranes are carefully arranged according to the same pattern in all of the organelles in the cell.

100: Dissecting the role of plasmepsin II and III in piperaquine resistant P. falciparum lines

Bopp, Selina (Harvard T.H.Chan School of Public Health); *Summers, Robert (Harvard T.H. Chan School of Public Health); Walsh, Breanna (Harvard T.H. Chan School of Public Health); Volkman, Sarah (Harvard T.H. Chan School of Public Health); Wirth, Dyann (Harvard T.H. Chan School of Public Health)*

The spread of artemisinin (ART) resistance makes partner drugs used in ART Combination Therapies more vulnerable to emerging drug resistance. Indeed, recent evidence indicates loss of efficacy of the partner drug piperaquine (PPQ) in Southeast Asia. We have previously shown a bimodal growth response to increasing PPQ concentrations in PPQ resistant P. falciparum isolates from Cambodia. Indeed, resistant parasites exposed to high PPQ concentrations were better able to survive than parasites exposed to lower drug levels. To quantify the bimodal response, we chose the area under the curve (AUC) instead of the conventional half-maximal effective concentration (EC50) used in drug assays. We confirmed a general association of high AUC values with increased plasmepsin II and III copy numbers. After subcloning a single Cambodian isolate, we obtained isogenic lines that varied only in their plasmepsin II and III copy numbers but not in the rest of the genome such as pfcrt sequence polymorphisms or mdr1 copy numbers assessed by whole genome sequencing. The line with a single copy of plasmepsin II/III has a low AUC while the other line with the duplication shows higher protein expression and higher AUC. As plasmepsin knockout (KO) lines show increased sensitivity to PPQ in a 3D7 background we generated plasmepsin II, plasmepsin III and plasmepsin II/III combination KO lines in these two isogenic isolates to confirm the association in this resistant background. Preliminary data suggests that a reduction in either plasmepsin II or III decreases the AUC compared to the parental line.. We will use these lines to further characterize the role of plasmepsin II and III in PPQ resistance.

101: Engineering a self-targeting entry inhibitor for vectored malaria prophylaxis

Srinivasan, Prakash (Johns Hopkins Malaria Research Institute, Dept. Molecular Microbiology and Immunology)

A critical step in Plasmodium falciparum (Pf) host cell invasion is the formation of a tight junction between the parasite and the host cell, which provides a firm anchor to facilitate parasite entry. This step requires interaction between the micronemal protein AMA1 and RON2, a component of the parasite secreted RON receptor complex. This interaction is also required for efficient Pf sporozoite invasion of hepatocytes. This is a unique example in host-pathogen interactions where both ligand and receptor are provided by the parasite for successful host cell entry. We recently showed that a 49 amino acid region of RON2 (termed RON2L) is sufficient to bind AMA1 and block invasion, thereby acting as a potent entry inhibitor. RON2L and its binding pocket in AMA1 is conserved within each Plasmodium species and points to an evolutionarily preserved “lock-and-key” mechanism designed to prevent changes. We reasoned that targeting this interaction can be an effective antimalarial strategy as it would make it harder for the parasites to develop resistance. To do this, we are developing a novel gene therapy approach using adeno-associated virus (AAV) to endogenously express the entry inhibitor RON2L masked as a human Ig fusion protein (RON2L-Ig). We show that the entry inhibitor expressed by AAV transduction is produced as a correctly folded, stable fusion protein and potently neutralized P. falciparum merozoites in vitro. We are using a in vivo rodent malaria model and a P. falciparum humanized mouse model to evaluate protection against infection and disease. Our data indicate that self-targeting this critical invasion pathway used by both the transmitting and disease causing forms of Plasmodium could provide an effective prophylaxis tool that can augment current malaria elimination efforts.

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

Hovel-Miner, Galadriel; *Sciascia, Nicholas; Quinn, McKenzi; Wu, Wi; Nussenzweig, Andre*

1- Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD 20892, USA
2- Department of Microbiology, Immunology, and Tropical Medicine, The George Washington University, Washington, D.C. 20037, USA

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

103: Synthesis Of Sterols And Sphingolipids Protect Leishmania Against Membrane Perturbing Agents, Osmostress And Starvation

MOITRA, SAMRAT (Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409)*; XU, WEI (Department of Molecular Microbiology, Center for Women's Infectious Disease Research, Washington University School of Medicine, Saint Louis, MO 63110); ZHANG, KAI (Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409)*

Within the mid gut of sand fly, Leishmania promastigotes need to colonize, proliferate and differentiate while facing a number of challenges including sand fly immunity, nutrient competition, potential microbial toxins and osmolality changes associated with sugar and blood meal digestion. Sterols and sphingolipids are key components of the plasma membrane and regulate diverse processes including membrane stability, ligand-receptor interaction, and vesicular trafficking. To decipher the protective role of these lipids in Leishmania, we examined the sensitivity of ergosterol and sphingolipid mutants to membrane perturbation agents, bacterial toxins and osmotic stress. Our findings indicate that alteration of lipid synthesis can make Leishmania extremely vulnerable to membrane stress and nutrient deprivation. Future work will elucidate: 1) how sterol/sphingolipid synthesis affects the composition and physiology of plasma membrane, and 2) how lipids in plasma membrane affect the transmission of Leishmania parasites in the sand fly.

104: Novel uses of familiar reagents: A method for enrichment of ring stage Plasmodium falciparum

Brown, Audrey C. (University of Virginia); *Guler, Jennifer (University of Virginia)*

The lack of enrichment methods for ring-stage P. falciparum leaves a large red blood cell (RBC) to parasite ratio in ring samples. Common saponin treatment disrupts RBC membranes releasing cytosolic contents. However, the overall RBC membrane structure remains intact allowing membranes to pellet and persist in samples. Our recent work demonstrates RBC membranes contribute heavily to the metabolome of ring-stage samples and may lead to noise that masks interesting parasite phenotypes. While uninfected RBC (uRBC) controls can limit this noise, they cannot eliminate it. We present a ring-stage enrichment method that reduces the RBC membrane to parasite ratio up to 20-fold. We predict enrichment of ring P. falciparum will decrease noise in the metabolome by reducing host contributed metabolic variation. Our method utilizes the pore-forming toxin, Streptolysin-O (SLO). SLO preferentially lyses uRBCs while leaving infected RBCs (iRBCs) primarily intact (84% uRBC lysis, 14% iRBC lysis in 27.4 ug/ml SLO; N=6). SLO treatment generates two populations: a lysed population largely derived from uRBCs and an intact population enriched for ring-stage parasites (we achieve enrichment up to 78.5% parasitemia). The physical differences between these populations are then exploited using a Percoll gradient to separate the intact, parasite enriched fraction from the lysed fraction. We demonstrate our SLO-Percoll (SLOPE) method is non-toxic by reculturing enriched fractions (after dilution to parasitemias <0.5%). Growth rate of recultured SLOPE-enriched fractions shows no difference compared to controls. Additional method validation includes targeted metabolomics of SLOPE-enriched samples compared to unenriched samples. This design allows accurate detection/quantification of host contributed metabolites. SLOPE enrichment is scalable, rapid (30min), and non-toxic making it ideal for use upstream of metabolomics analyses.

105: Optimized CRISPR/Cas9 protocols permit new gene editing applications in P. falciparum

Crater, Anna (NIAID, National Institutes of Health); *Desai, Sanjay (NIH NIAID)*

The CRISPR/Cas9 system has simplified gene editing in Plasmodium falciparum, facilitating advances in biology and antimalarial therapeutics; it requires only the expression of a Cas9 endonuclease and single guide RNAs (sgRNA) to direct target cleavage. We used a sensitive kinetic Nanoluc reporter, conditional Cas9 expression, and a promoter trap strategy to optimize CRISPR/Cas9 transfection in this pathogen. Our quantitative kinetic measurements permitted systematic evaluation of a series of tracer modifications; they also provided threshold on- and off-target efficiency scores that reduce trial-and-error experimentation. We determined that the parasite RNA polymerase III (RNAPIII) promiscuously accepts U6 promoters from other Plasmodia spp., allowing replacement of the large and integration-prone P. falciparum U6 promoter with a smaller and equally effective P. berghei version; efficacy relative to the heterologous T7 RNA polymerase will also be presented. Importantly, these studies revealed that efficient transcription termination with the parasite RNAPIII requires a 9-10 bp poly-T signal, in contrast to the 6 bp signal typical of yeasts and higher eukaryotes. Parasite outgrowth after transfection was inversely related to sgRNA on-target efficiency, implicating rate-limited genomic repair; we confirmed this model by overexpressing the parasite Rad51 enzyme needed for homologous recombination. Finally, we examined key variables in transfection usually left to personal preference (timing and concentration of drug selection, blood addition, plasmid amount, etc.). Our optimized conditions yielded essentially complete gene modification within 8 days of transfection. Several novel applications of CRISPR that allow multiple genome edits were enabled and will also be presented. This study provides definitive guidance for efficient CRISPR/Cas9 transfection and enables new applications for basic and translational malaria research.

106: The Role Of Encephalitozoon Hellem Sporoplasm Protein 1 (EhSPP1) In Microporidia Development And Host Cell Invasion

Han, Bing (Albert Einstein College of Medicine); *Weiss, Louis (Albert Einstein College of Medicine)*

Microsporidia are opportunistic zoonotic intracellular pathogens that are able to infect a wide variety of hosts. They process a unique, highly specialized invasion organelle termed the polar tube. When exposed to the correct conditions the polar tube rapidly discharges out of the spore and delivers the sporoplasm to the host cell. Our group has previously determined that polar tube proteins PTP1 and PTP4 interact with host cell surface proteins. Our model of invasion is that the polar tube pushes into the host cell creating a protective microenvironment, the invasion synapse, into which the sporoplasm is then extruded. We are interested in understanding the invasion event in this synapse, e.g. how the sporoplasm interacts and invades the host cell within this microenvironment. We have recently identified a novel sporoplasm protein 1 (SPP1) from E. hellem using a proteomic approach. Antibodies to SPP1 were produced using purified recombinant SPP1 (rSPP1). IFA demonstrated that EhSPP1 localized to the surface of the extruded sporoplasm. In addition, rSPP1 bound to the host cell surface and the parasitophorous vacuole membrane (PVM) in infected cells. SPP1 may be involved in the interaction of the extruded sporoplasm with a host cell surface receptor in the invasion synapse and in the localization of immature replicating stages to the PVM. As the spore wall forms, a loss of SPP1 host cell membrane interaction would allow mature spores to move to the internal region of the parasitophorous vacuole. To identify potential host cell proteins interacting with EhSPP1 a co-immunoprecipitation combined with the proteomic analysis is in progress. [Supported by NIH AI124753 (LMW)].

107: Malaria Parasites Require a Divergent Heme Oxygenase for Apicoplast Maintenance

***Nasamu, Sebastian**; Beck, Josh; Caaveiro, Jose; Niles, Jacquin; Goldberg, Daniel; Sigala, Paul (University of Utah)*

During blood-stage growth, Plasmodiumparasites import and digest up to 80% of host hemoglobin and liberate vast amounts of heme. Plasmodiumspecies lack a canonical heme degradation pathway, and most of this liberated heme is sequestered in the parasite digestive vacuole as hemozoin. Nevertheless, a small amount of host heme appears to escape this vacuole and partition into the cellular matrix, where it can be utilized within parasite organelles. Heme metabolism within the parasite is coordinated with overall cellular and organellar division, but the regulatory mechanisms that govern blood-stage Plasmodiumdevelopment remain sparsely understood. We have uncovered that P. falciparumparasites express a divergent heme oxygenase (PfHO) targeted to the apicoplast, a non-photosynthetic plastid that retains a small genome and houses core metabolic pathways. Structural and biochemical studies confirm that PfHO retains the canonical HO fold but lacks a critical heme-coordinating His residue and thus binds but cannot degrade heme.

In-parasite studies reveal that PfHO is proteolytically processed upon import into the apicoplast but surprisingly retains most of its positively charged transit peptide. This N-terminal domain confers an ability to bind DNA that is antagonized by heme binding. We used CRISPR/Cas9 to tag PfHO for conditional expression with the aptamer/TetR-DOZI system, and its knockdown reveals an essential role in apicoplast maintenance and thus parasite viability. Specifically, PfHO knockdown prevents apicoplast RNA levels from increasing in mature parasites, suggesting a failure to initiate transcription of the apicoplast genome. We propose that P. falciparumparasites have repurposed PfHO to function as a heme-dependent regulator of apicoplast transcription.

We hypothesize that PfHO functions as a cryptic sigma factor to recruit RNA polymerase to the apicoplast genome to initiate transcription, then turns off transcription in response to increased labile host heme as mature parasites prepare for erythrocyte egress. This divergent function of PfHO provides an elegant adaptation by P. falciparumparasites to the unique, heme-rich environment of erythrocytes and may provide a new target for antimalarial therapeutics.

108: TgCentrin2 regulates invasion and proliferation by the human parasite Toxoplasma gondii

Leung, Jacqueline M. (Indiana University Bloomington); *Liu, Jun (Indiana University Bloomington); Wetzel, Laura (Indiana University Bloomington); Murray, John M. (Indiana University Bloomington); Hu, Ke (Indiana University Bloomington)*

Centrins are EF-hand containing proteins ubiquitously found in eukaryotes and are key components of centrioles/basal bodies as well as certain contractile fibers. We previously identified three centrins in the human parasite Toxoplasma gondii, all of which localized to the centrioles as predicted.

However, one of them, TgCentrin2, is also simultaneously targeted to ring-shaped structures at the apical and basal ends of the parasite, as well as to multiple annuli found at the junction between the apical cap and flattened IMC vesicles. The role(s) that TgCentrin2 plays in each of these locations is unknown. Here we report the functional characterization of TgCentrin2 in the parasite lytic cycle. After multiple unsuccessful attempts to knock out the TgCentrin2 gene, we designed a conditional knockdown method that uses a combination of transcriptional and protein stability control to achieve tight regulation of TgCentrin2 levels in the parasite. We discovered that under knockdown conditions, there is an ordered, sequential loss of TgCentrin2 from its multiple compartments, suggesting differential protein turnover rates and/or incorporation kinetics. The knockdown of TgCentrin2 also resulted in severe defects in several aspects of the T. gondii lytic cycle, including invasion, partly due to a decrease in the secretion of adhesins. We also found that in a subsequent round of the lytic cycle, sustained knockdown of TgCentrin2 led to defects in intracellular replication. We are currently characterizing these phenotypes in more detail to pinpoint the functions of TgCentrin2 in the parasite.

109: Dissecting the role of RPA1 in the Leishmania major response to DNA replication stress

Bastos, Matheus Silva (Ribeirão Preto Medical School, University of São Paulo)*; Silva, Gabriel Lamak Almeida (Ribeirão Preto Medical School, University of São Paulo); Virgilio, Stela (Ribeirão Preto Medical School, University of São Paulo); Damasceno, Jeziel Dener (The Wellcome Trust Centre for Molecular Parasitology); McCulloch, Richard (The Wellcome Trust Centre for Molecular Parasitology); Tosi, Luiz Ricardo Orsini (Ribeirão Preto Medical School, University of São Paulo)*

The eukaryotic Replication Protein A (RPA) complex is composed by three distinct subunits and constitutes a key factor in many processes of the DNA metabolism. RPA1 is the largest subunit of the complex and bears most of the domains involved in the RPA-DNA interaction. In trypanosomatids, the RPA1 homolog has been shown to participate in the response to replication stress and diverge from other RPA1 subunits by lacking one of the domains involved in the interaction with partner proteins and complexes. To better understand RPA function in Leishmania majorwe used the CRISPR/cas9 system to generate a cell line that expresses a N-terminal tagged version of RPA1 from the endogenous locus. The 3xMyc-RPA1 cell line has both alleles of the RPA1 locus replaced and the expressed 3xMyc-RPA1 is localized exclusively in the nuclear compartment. The growth profile and the pattern of response to different concentrations of replication stress-inducing drug, hydroxyurea (HU), was comparable to that observed for wild type cells. We also found that 3xMyc-RPA1 is enriched in the chromatin fraction is response to replication stress. These findings suggested that endogenous tagging did not affect RPA1 function. Alternative cell lines were also generated and include a heterozygous line expressing a 12xMyc C-terminal-tagged RPA1. A cell line expressing a functional NeonGreen N-terminal-tagged RPA1 was also generated and can be used in live cells studies. These cell line are currently being used to characterize Leishmania RPA complex and the parasite response to replication stress. Supported by FAPESP (16/18192-1; 16/50500-2).

110: Characterization of the chromatin binding profile of RPA and 9-1-1 complexes of Leishmania major in response to replication stress

Virgilio, Stela (Ribeirão Preto Medical School, University of São Paulo)*; Bastos,Matheus Silva (Ribeirão Preto Medical School, University of São Paulo); Damasceno, Jeziel Dener (The Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow); McCulloch, Richard (The Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow); Tosi, Luiz Ricardo Orsini (Ribeirão Preto Medical School, University of São Paulo)*

The eukaryotic ATR pathway orchestrates the cellular response to a wide variety of DNA injuries including replication stress and double strand breaks, and is highly dependent on early signaling events. The detection of accumulated single-stranded DNA (ssDNA), which is a common effect of genome injuries, sets the stage for ATR recruitment and activation leading to the stalling of cell cycle progression, protection of DNA replication forks and recruitment of DNA repair activities. The tripartite complexes RPA (RPA1-RPA2-RPA3) and 9-1-1 (RAD9-RAD1-HUS1) participate in the detection of ssDNA and activation of ATR kinase, respectively. We have shown that Leishmania major 9-1-1 complex and RPA subunit RPA1 participate in the response to replication stress, suggesting a conserved ATR pathway in these deep-rooting eukaryotes. To further characterize this pathway in Leishmania and identify RPA and/or 9-1-1-enriched loci in response to replication stress, we used chromatin immunoprecipitation (ChIP) assay of L. major RPA1- or HUS1-associated loci. ChIP assays were carried out in endogenous-tagged RPA1 and HUS1 cell lines (3xMyc-RPA1 and HUS1-12xMyc). The growth pattern and cell cycle progression profile of these cell lines were comparable to that of wild-type cells. To establish the ChIP protocol, we fully optimized the protocol from sample preparation to DNA fragmentation and immunoprecipitation. Western blot analyses showed that both RPA1 and HUS1 were efficiently immunoprecipitated from samples submitted to different levels of replication stress and the multi-copy spliced leader gene was probed to validate the ChIP assays. Large-scale sequencing and analysis of ChIP products are currently underway to identify the profile of the parasite's response to different DNA replication stress conditions. Supported by FAPESP (16/18191-5; 16/18192-1; 16/50500-2).

111: Investigating The Interactome Of The Toxoplasma Gondii CYST Wall

Tu, Vincent (Albert Einstein College of Medicine)*; Sugi, Tatsuki; Tomita, Tadakimi (Albert Einstein College of Medicine); Yakubu, Rama (Albert Einstein College of Medicine); Han, Bing (Albert Einstein College of Medicine); Ma, Yanfen (Albert Einstein College of Medicine); Mayoral, Joshua (Albert Einstein College of Medicine); Williams, Tere (Albert Einstein College of Medicine); Weiss, Louis (Albert Einstein College of Medicine)*

Toxoplasma gondii, an opportunistic pathogen, infects more than 1 billion people worldwide. The majority of infected individuals harbor this intracellular parasite as a latent infection with cysts (containing bradyzoites) being found in the central nervous system. However, these cysts can reactive, especially in immune compromised hosts, leading to active retinochoroiditis or encephalitis. Currently, there are no drugs that can eliminate T. gondii cysts from infected patients. A characteristic of the latent (cyst) stage of T. gondii is a thick cyst wall that forms underneath the parasitophorous vacuole membrane. Previously, our laboratory discovered cyst wall protein 1 (CST1) that is an integral protein in the cyst wall which maintains the structural integrity and morphology of the cyst. Bradyzoite pseudokinase 1 (BPK1) and microneme-adhesion-repeat domain containing protein 4 (MCP4) have also been demonstrated to localize to the cyst wall. To understand how these and other proteins interact with one another and to identify new proteins in the cyst wall, i.e. Cyst Wall Proteins (CWPs), we have tagged several CWPs with a promiscuous biotin ligase (BirA) and determined their interacting partners by purifying biotinylated proteins from the parasite. Within the cyst wall interactome, we have uncovered previously described CWPs, dense granule proteins (GRAs), and uncharacterized hypothetical proteins (putative CWPs). We validated the hypothetical proteins to be novel protein components of the cyst wall using HA tags and have characterized the role of these new CWPs in the formation of the cyst wall.

These studies have provided the reagents and groundwork to understand cyst wall formation and should provide insights for approaches to eliminate latent parasites before reactivation [Supported by NIH grant AI134753 (LMW)].

112: A kinetic assay provides mechanistic insights into malaria parasite protein trafficking and insertion at the host membrane

Shao, Jinfeng (NIAID, NIH, Bethesda, MD, USA); *Saggu, Gagandeep Singh; Desai, Sanjay*

Many Plasmodium falciparum membrane proteins are exposed at the host cell surface, mediating critical functions such as cytoadherence and nutrient acquisition. How these proteins traffic to and insert in the host membrane is largely unknown. Because mature erythrocytes are unable to mediate this process, the required machinery must be parasite-derived and established de novo. Mechanistic insights have been limited because heterologous proteins have been difficult to target to the host membrane and because conventional reporter proteins yield signals before insertion. We addressed these problems with a split Nanoluc reporter where a fragment was inserted in a small extracellular loop of CLAG3, a surface protein linked to the nutrient uptake channel PSAC. The addition of a 27-residue tag within this extracellular loop did not adversely affect CLAG3 export or insertion at the host membrane. A separate transfectant line expressing a CLAG3 protein with this tag but without downstream protein sequences failed to insert in the host membrane, implicating a role for the CLAG3 C-terminus in trafficking. Kinetic studies with these reporter parasites revealed that CLAG3 trafficking to the host membrane takes > 24 h after invasion and that PSAC activation depends on host membrane insertion of this protein. Finally, our reporter assay has been miniaturized for high-throughput screens that aim to find inhibitors of parasite protein insertion at the host membrane. These studies should uncover the trafficking mechanisms for exposed parasite antigens, provide insights into activation of nutrient channels at the host membrane, and may guide development of novel therapies that interfere with host cell remodeling.

113: Altered Morphology of the Digestive Vacuole Disrupts Integrity of the Endolysosomal System in Toxoplasma gondii

Thornton, Brock (Clemson University)*; Teehan, Paige (Pennsylvania State University); Cochrane, Christian (Clemson University); Floyd, Katherine (Clemson University); Bergmann, Amy (Clemson University); Dou, Zhicheng (Clemson University)*

Toxoplasma gondii is an obligate intracellular protozoan parasite. The digestive vacuole, termed the Vacuolar Compartment/Plant-Like Vacuole (VAC/PLV), plays multiple functions in both acute and chronic Toxoplasma infections. An ortholog of the malarial chloroquine resistance transporter (TgCRT) was reported to span the membrane of the VAC. To understand its role during Toxoplasma infection, the TgCRT gene was genetically ablated. The resulting mutant, ?crt, increased the volume of its VAC ~15-fold. The swollen VAC disrupted the integrity of the parasite's endolysosomal system, thereby resulting in reduced microneme secretion and invasion. Moreover, the ?crt strain had significantly decreased acute virulence compared to WT parasites. Based on these phenotypic observations, we hypothesize that TgCRT transports small solutes derived from decomposed protein by serving as a polyspecific nutrient exporter and regulates the size of the VAC. To test this hypothesis, transcriptome sequencing was performed to measure global gene transcription changes in the WT and ?crt strains. The transcript levels of four proteases were decreased greater than 1.5-fold in ?crt compared to WT. Using quantitative PCR and endogenous epitope tagging, three proteases were confirmed to have reduced transcripts in ?crt and were shown to reside within the endolysosomal system. Additionally, the protein levels of two known VAC luminal proteases, cathepsin L and B-like proteases (TgCPL and TgCPB), were decreased in ?crt parasites. Chemical and genetic suppression of proteolysis within the VAC also reduced the size of the VAC. Collectively, these studies determined the mechanism by which TgCRT controls the size of the digestive vacuole, which provides insight on the native function of TgCRT and the regulation of the physiology and function of the VAC in Toxoplasma.

114: TIMP-1 promotes hypermigration of T. gondii-infected dendritic cells through CD63 / ITGB1 / FAK signaling

Olafsson, Einar B. (Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute); *Barragan, Antonio (Stockholm University, MBW)*

Dendritic cells (DCs) infected by Toxoplasma gondii rapidly acquire a hypermigratory phenotype that promotes systemic parasite dissemination by a 'Trojan horse' mechanism in mice. Upon invasion, the parasite orchestrates a migratory program characterized by cytoskeletal reorganization and high-speed amoeboid migration of invaded DCs. Recently, we showed that T. gondii-infected DCs undergo mesenchymal to amoeboid transition (MAT) characterized by abrogated degradation, of extracellular matrix in vitro and secretion of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1). However, the role of TIMP-1 in the amoeboid migration mode of immune cells remains unknown. Using a short hairpin RNA (shRNA) gene silencing approach, we show that secreted TIMP-1 and its putative receptor CD63 are required for hypermotility of T. gondii-infected DCs. Signal transduction occurred via the CD63-ß1 integrin (ITGB1) complex. Further, gene silencing of focal adhesion kinas (FAK) or pharmacological antagonism of FAK and associated kinases Src and Pi3k abolished hypermotility. The present work identifies the TIMP-1/ CD63/ ITGB1/ FAK signaling axis in DCs, and shows how T. gondii utilizes TIMP-1 to drive high-speed amoeboid migration of infected DCs.

115: Critical role of phosphorylation of the malarial cGMP-dependent protein kinase (PKG)

Kousis, Konstantinos (The Francis Crick Institute); *Withers-Martinez, Chrislaine (The Francis Crick Institute); Baker, David A. (London School of Hygiene & Tropical Medicine); Blackman, Michael J. (The Francis Crick Institute and London School of Hygiene & Tropical Medicine)*

Cyclic guanosine monophosphate (cGMP) regulates important signalling functions in most eukaryotic organisms. In the malaria parasite, all cGMP signalling is mediated through a single cGMP-dependent protein kinase (PKG). Genetic and chemical approaches have shown that this enzyme has essential roles in late liver stage development, merozoite egress, gametogenesis, ookinete conversion and motility as well as sporozoite motility and infection.

However, many details of PKG function remain unclear. In an effort to better understand the role of PKG in Plasmodium falciparum blood stages we have used the DiCre system and a novel approach of introducing multiple lox sites into a single artificial intron allowing the generation of two distinct lines in one recombination event. This approach has been used to create in parallel a conditional knock-out of PKG and a recodonised wild type PKG. Extending this approach, we have developed parasite lines in which we can conditionally replace the genomic PKG gene with mutant alleles that are enzymatically inactive, or partially or completely refractory to phosphorylation. Egress assays with the mutant lines have shown that even pharmacologically enhanced cGMP levels cannot bypass the absence of PKG or activate non-phosphorylated enzyme, with the exception of a single tyrosine mutant. Our results show that phosphorylation of PKG is likely critical for its function. Efforts are currently underway to identify autophosphorylation sites and examine how mutation of these sites affects the activity and function of PKG in vitro and in vivo.

116: Plasmodium falciparum ubiquitin transferase, a novel putative quinine resistance marker

Jankowska-Döllken, Monika (Heidelberg University Hospital)*; Sanchez, Cecilia (Heidelberg University Hospital); Lanzer, Michael (Heidelberg University Hospital)*

Drug resistant Plasmodium falciparum malaria is spreading in tropical and subtropical areas, causing over 400 000 deaths annually. Understanding the factors that lead to the development and spread of drug resistance is necessary to design optimal prevention and treatment strategies. A HECT ubiquitin ligase, termed Plasmodium falciparum ubiquitin transferase (PfUT), is a novel candidate gene for multifactorial resistance to the oldest antimalarial drug, quinine. How PfUT affects quinine responses is, however, currently unclear. PfUT was shown to localize to the parasites's ER/Golgi complex, but its functional role still needs to be validated. Here, we have characterized a conditional knockdown of pfut gene in P. falciparum, generated by incorporation of the glucosamine inducible glmS ribozyme in the 3' untranslated region of PfUT, using the CRISPR/Cas9 genome editing technology. We found that the insertion of glmS sequence led to increased pfut transcript levels, in the absence of glucosamine. As expected, upon glucosamine treatment, the pfut transcript levels declined. Moreover, we observed that the PfUT-glmS line showed an extended duration of asexual life cycle, due to an increased length of the S phase, regardless of the presence or absence of glucosamine.

Interestingly, the PfUT-glmS parasites were also more susceptible to quinine, already in the absence of glucosamine. Glucosamine addition further enhanced the effect to even lower response levels. Further studies regarding validation of PfUT's association with resistance to quinine and the identification of its biological targets are currently ongoing.

117: An apical motility switch in Toxoplasma gondii

Brown, Kevin M. (Washington University in St. Louis)*; Sibley, L. David (Washington University in St. Louis)*

Apicomplexans have adapted cyclic GMP signaling for cell to cell transmission. Although much is known about the essential kinases (PKGs) that relay the cGMP signal, it is not clear how cGMP signaling is initiated in these parasites. In other eukaryotes, GTP is converted to cGMP by guanylate cyclases (GCs) that are regulated by domains that sense nitric oxide or peptide hormones.
Previous studies in Plasmodium demonstrated that apicomplexan GCs are active and likely essential, but lack known regulatory domains. Instead, apicomplexan GCs possess an N-terminal P-type ATPase domain of unknown function. Here we report that Toxoplasma encodes a single GC that localizes to the plasma membrane proximal to the apical cap region of the IMC. Using an auxin degron system, we found that loss of TgGC completely blocked lytic growth due to defective motility, invasion and egress. These motility defects were explained by a profound defect in microneme secretion, phenocopying loss of TgPKG. Cell-permeable cGMP reversed the defects in microneme caused by loss of TgGC, but not TgPKG, indicating that the primary role of TgGC is to produce cGMP. Using genetic complementation, we performed a functional domain analysis of TgGC and found that the P-type ATPase and GC domains must be catalytically active and co-expressed in a linked state. We speculate that the P-type ATPase domain functions as a regulatory domain for the GC domain, which may occur through phospholipid flipping or ion transport at the plasma membrane. To determine whether TgGC is also important for host infection, we adapted the auxin degron system for use in mice by introducing the auxin receptor into Me49 FLuc. We found that TgGC-mAID could be depleted in parasites by administering auxin orally to mice, protecting all mice from lethal toxoplasmosis. Following auxin knockdown, parasites failed to disseminate from the site of infection and parasite burdens rapidly declined based on BLI imaging. Our findings suggest that the dual domain structure in TgGC is an essential regulatory feature that may extend to other apicomplexans and related protists.

118: Genome-wide Screening of Copy Number Variation in Plasmodium falciparum Clinical Isolates

Assisi, Christina (Nanyang Technological University)*; Bozdech, Zbynek (Nanyang Technological University)*

Genome-wide Screening of Copy Number Variation in Plasmodium falciparum Clinical IsolatesChristina Assisia, Zbynek BozdechaaSchool of Biological Sciences, Nanyang Technological University, SingaporeGene Copy Number Variation (CNV) has been associated with important phenotypes pertaining Plasmodium falciparum adaptive evolution towards its hosts and environmental perturbations.We use array Comparative Hybridization to screen for CNVs in Plasmodium falciparum clinical isolates obtained during Tracking Resistance to Artemisinin Collaboration (TRAC) study. Our preliminary result shown detection of formerly established CNV markers of drug resistance such as pmdr1 and plasmepsin II, as well as few other novel CNVs which has not been fully characterized. Further characterization of novel CNVs would be valuable for malaria surveillance and may provide insight for development of new and effective malaria treatment. The CNV finding in this study would reflect the genetic adaptability of Plasmodium falciparum in overcoming various perturbations and deepen our current understanding of its biological significance in the clinical isolates.

119: A molecular basis for the control of local Toxoplasma gondii in wild South American Mus musculus populations

Alvarez, Catalina (Instituto Gulbenkian de Ciencia)*; Müller, Urs (University of Cologne); Campos, Claudia (Instituto Gulbenkian de Ciência); Steinfeldt, Tobias (University of Freiburg); Howard, Jonathan (Instituto Gulbenkian de Ciencia)*

Immunity-related GTPases (IRGs) are IFN?-inducible genes of a cell-autonomous resistance system that protects the mouse host from avirulent Toxoplasma gondii strains. However, virulent strains of the parasite can inactivate IRG proteins and kill laboratory mice within days. Virulence in T. gondii kills mice before encystment and interrupts the parasite life cycle but certain highly polymorphic IRG proteins found in wild-derived Eurasian mice confer resistance even against virulent parasite strains. By deletion of the tandem IRG protein Irgb2-b1 in cells from the resistant mouse strain CIM, we show that Irgb2-b1 is responsible for the resistance, via direct binding to the T. gondii virulence factor ROP5 isoform B.The interaction between South American (SA) T. gondii strains and Eurasian mice in nature is only 500 years old and virtually all SA T. gondii tested are virulent for laboratory mice and some Eurasian wild-derived strains. We show that this virulence is due to polymorphism present in the ROP5 isoforms of some SA T. gondii strains since they cannot be targeted by specific alleles of the Irgb2-b1 gene.We show Brazilian wild mice have an exceptionally high prevalence of a specific Eurasian Irgb2-b1 allele, which is unlikely due to a founder effect. Mouse cells carrying this particular allele are more efficient at controlling SA T. gondii strains in vitro than are mouse cells with other Eurasian alleles. The highly prevalent Irgb2-b1 allele has therefore probably been selected from the IRG allele pool imported by Eurasian mouse immigrants because it provides resistance against local SA T. gondii strains.

120: New Permeation Pathways in the host red cell during Plasmodium falciparum gametocytogenesis: greater than expected

Bouyer, Guillaume (CNRS UMR8227, Station Biologique de Roscoff, Sorbonne Université, Labex GR-Ex, France)*; Barbieri, Daniela (INSERM U1016, CNRS UMR 8104, Université Paris Descartes, Labex GR-Ex, France); Marteau, Anthony (INSERM U1016, CNRS UMR 8104, Université Paris Descartes, Labex GR-Ex, France); Roman, Diana (IRD, Université Paris Descartes, Labex GR-Ex, France); Dupuy, Florian (INSERM U1016, CNRS UMR 8104, Université Paris Descartes, Labex GR-Ex, France); Sissoko, Abdoulaye (IRD, Université Paris Descartes, Labex GR-Ex, France); Siciliano, Giulia (Istituto Superiore di Sanita, Italy); Gomez, Lina (INSERM U1016, CNRS UMR 8104, Université Paris Descartes, Labex GR-Ex, France); Alano, Pietro (Istituto Superiore di Sanita, Italy); Martins, Rafael (CNRS - 5290, IRD 224 - University of Montpellier (UMR 'MiVEGEC'), Montpellier, France); Lopez-Rubio, José-Juan (CNRS - 5290, IRD 224 - University of Montpellier (UMR 'MiVEGEC'), Montpellier, France); Clain, Jérome (IRD, Université Paris Descartes, Labex GR-Ex, France); Duval, Romain (IRD, Université Paris Descartes, Labex GR-Ex, France); Egée, Stéphane (CNRS UMR8227, Station Biologique de Roscoff, Sorbonne Université, Labex GR-Ex, France); Lavazec, Catherine (INSERM U1016, CNRS UMR 8104, Université Paris Descartes, Labex GR-Ex, France)*

It has long been known that following malaria parasite invasion, the infected red blood cell (iRBC) displays important alterations of its membrane properties, notably permeability and deformability. The activity of several endogenous transporters is modified, and New Permeation Pathways (NPPs) appear with properties unlike those of endogenous red cell transport system. These NPPs are essential for asexual parasites to ensure the transport of nutrients and waste products necessary for their replication and survival. The NPPs are active at the trophozoïte and schizont stages with the features of a weakly selective anion channel although their number and full molecular identity remains controversial.While the properties of NPPs have been extensively characterized in asexual parasite stages, only one study has focused on membrane permeability of gametocyte iRBCs, leading to the dogma that there are no NPPs activity all along gametocytogenesis.Using a diversity of techniques such as isosmotic lysis, electrophysiology and fluorescence tracer uptake, we show here that NPPs are still active in RBCs infected with immature sexual stages, although weaker than in asexual stages, and that this activity declines along the maturation of gametocytes from stage I to stage V. Regulation of this residual NPPs activity involves cAMP/PKA pathway, as it was already demonstrated for NPPs in asexual stages and for iRBCs deformability upon gametocytogenesis. In addition, our results suggest that NPPs contribute to the uptake of antimalarials and that NPPs slowdown in mature gametocyte stages may account for their resistance to several drugs.

121: A membrane associated PPM family protein phosphatase, PPM5C, regulates Toxoplasma gondii attachment to host cells

Yang, Chunlin (Indiana University School of Medicine)

Toxoplasma gondii, an obligate intracellular parasite, infects around one-third of the human population. Although a healthy person's immune system can quickly control acute infection of the parasites, individuals who have compromised or suppressed immune systems are susceptible to severe disease. The propagation of T. gondii is accomplished by repeated lytic cycles of the parasites, which consist of parasite attachment to the host cell, subsequent invasion, parasitophorous vacuole (PV) formation, replication inside the PV, and then egress from the host cell. This lytic cycle is delicately regulated by calcium-dependent reversible phosphorylation of the molecular machinery that drives invasion and egress is regulated by parasite specific kinases. While much progress has been made to elucidate the protein kinases and substrates central to parasite propagation, little is known about the relevant protein phosphatases. Accordingly, our research interest is to identify and determine the functions of the protein phosphatases involved in the lytic cycle of T. gondii. Thus, we performed a bioinformatics analysis of the T. gondii genome to identify protein phosphatases that would be predicted to associate with the parasite's pellicle since this is the location of the molecular machinery that drives invasion and egress. Four protein phosphatases that were predicted to potentially be membrane-associated were selected for endogenous gene tagging. We have determined that only PPM5C (TgGT1_281580), a PP2C family member, localizes to the plasma membrane of T. gondii. Disruption of PPM5C results in a slow propagation phenotype in tissue culture. Rate of propagation is a multi-factorial phenotype that is influenced by the efficiency of attachment, invasion, and egress as well as parasite division. Interestingly, parasites lacking PPM5C divide and undergo egress at a normal rate, but invasion assays showed that disruption of PPM5C resulted in deficient entry into host cells. Further characterization revealed that lack of PPM5C did not impact invasion efficiency of parasites but moderately defect parasites' ability to attach to host cells. Therefore, our data suggest that PPM5C is a key regulator of proteins key to host cell attachment. Current studies are focused on identifying substrates of PPM5C and determining the underlying mechanism.

122: The apicomplexan membrane-trafficking system: gains, losses, and novel features

Klinger, Christen M. (University of Alberta); *Dacks, Joel B. (University of Alberta); Meissner, Markus (Ludwig Maximilian University Munich)*

Apicomplexa are obligate intracellular parasites that cause significant morbidity, mortality, and economic hardship worldwide. Host cell invasion involves secretion of specialized organelles, including micronemes and rhoptries, which may be homologous to endolysosomes. In addition, most apicomplexans possess an apicoplast, a non-photosynthetic complex plastid involved in parasite metabolism. Disrupting processes involved in the biogenesis of, trafficking to, or partitioning of these organelles into daughter cells during division proves fatal by disrupting one or more steps of the parasite life cycle. Membrane trafficking of protein and lipid components between distinct organelles require combinatorial interactions of multi-gene family members, in which each member performs a similar function at specific locations to ensure the specificity of trafficking pathways. Hence, it can be expected that apicomplexans should either encode additional paralogues of each family, or re-purpose existing family members whose original function has been lost, in order to support trafficking to organelles such as micronemes, rhoptries, and the apicoplast. Surprisingly, previous studies found no additional trafficking machinery; conversely, a pattern of lineage-specific loss predominated for each family investigated. Thus, we have undertaken a large-scale bioinformatic analysis incorporating a novel phylogenetic pipeline to characterize the trafficking complement of Apicomplexa and their close relatives. Altogether, we identify further cases of loss of pan-eukaryotic factors, but also the existence of previously undescribed paralogues in these lineages. Tagging and disruption studies reveal important roles for some of these factors in Toxoplasma gondii, suggesting that innovation of trafficking machinery played an important role in apicomplexan evolution.

123: Push and Pull: the role of actin in invasion.

Del Rosario, Mario (University of Glasgow - Wellcome Centre for Molecular Parasitology)*; Latorre-Barragan, Fernanda (University of Glasgow - Wellcome Centre for Molecular Parasitology); Das, Sujaan (University of Glasgow - Wellcome Centre for Molecular Parasitology); Pall, Gurman (University of Glasgow - Wellcome Centre for Molecular Parasitology); Stortz, Johannes Felix (University of Glasgow - Wellcome Centre for Molecular Parasitology); Whitelaw, Jamie (Beatson Institute for Cancer Research); Periz, Javier (University of Glasgow - Wellcome Centre for Molecular Parasitology); Lemgruber, Leandro (University of Glasgow - Wellcome Centre for Molecular Parasitology); Meissner, Markus (Ludwig-Maximilians-University of Munich)*

Invasion by apicomplexan parasites such as Plasmodium falciparum and Toxoplasma gondii into host cells requires the establishment of a ring-like junctional structure that bridges the parasite and host cell plasma membrane. Due to the force required by the parasite to go through this junction ring during invasion, the parasite suffers temporal deformations to squeeze through; a behaviour also reported for mammalian cancerous migrating cells. Recent findings demonstrate that parasites deficient of core components of the acto-myosin system are incapable of withstanding pressure exerted at the junction ring, leading to blebbing and collapse of the invading parasite. In this work, we hypothesised that the parasite nucleus represents a major obstacle for successful invasion and hence is a limiting factor for the speed and efficiency of invasion. A considerable large organelle, the nucleus presents a rigid structure that cannot go through the junctional ring with ease. We demonstrate that during T. gondii and P. falciparum invasion, F-actin bundles form around the nucleus connecting it to an F-actin ring close to the junctional ring, thus protecting the nucleus and allowing successful invasion. In addition, F-actin accumulates in the posterior end of the parasite, where it appears to be exerting force to push the nucleus through the junctional ring. Additionally, T. gondii mutants lacking core elements of the acto-myosin system start penetrating the host cell but fail to invade efficiently as soon as the nucleus reaches the junctional ring. Together, our results clarify the contribution of the acto-myosin system during invasion and lead to a new model for host cell invasion by apicomplexan parasites.

124: Ribozyme-Mediated, Multiplex CRISPR Gene Editing and CRISPRi in Plasmodium

Walker, Michael P. (Penn State University)*; Singh, Suprita (Pennsylvania State University); Josling, Gabrielle (Pennsylvania State University); Llinás, Manuel (Pennsylvania State University); Lindner, Scott (Pennsylvania State University)*

Ribozyme-Mediated, Multiplex CRISPR Gene Editing and CRISPRi in Plasmodium Michael P. Walker1, Suprita Singh1, Gabrielle Josling1, Manuel Llinás1, & Scott E. Lindner1 1Department of Biochemistry and Molecular Biology, Center for Malaria Research, Pennsylvania State University, University Park, PA. Functional characterization of genes in Plasmodium parasites typically relies on genetic manipulation to disrupt or modify a gene-of-interest. However, these approaches are limited by the time needed to generate transgenic parasites, as well as the risk of disrupting native regulatory elements with the introduction of exogenous sequences. Furthermore, some Plasmodium species, such as Plasmodium yoelii, a rodent-infectious species favored for rapid transfection and selection procedures, are significantly hampered by only having a single drug-selectable marker. To address these limitations, we have created a single-plasmid CRISPR/SpCas9 gene editing system that utilizes a Ribozyme-Guide-Ribozyme (RGR) sgRNA expression strategy. We demonstrate that this system efficiently generates both gene disruptions and fluorescent tag insertions using co-expressed sgRNAs to induce multiple double-strand breaks. Because this system expresses all editing elements from a single plasmid, gene modifications can be marker free and parasites can regain sensitivity to drug pressure upon curing the plasmid. We find that the number of sgRNAs used to edit the genome affects the editing outcomes, and that the common practice of using only one sgRNA can lead to undesired plasmid integration events. We also demonstrate that this RGR system can be adapted to edit Plasmodium falciparum by incorporating it into an existing dual plasmid CRISPR system. Lastly, we believe this RGR system can also be used for CRISPR interference (CRISPRi) by binding dCas9 to targets in the promoter/5' UTR of a gene-of-interest, and can produce an effective reduction in gene expression. This robust and flexible system should open the door for in-depth and efficient genetic characterizations in both rodent- and human-infectious Plasmodium species.

125: Rapid block of RNA splicing by chemical inhibition of analog-sensitive CRK9, a cyclin-dependent kinase essential in trypanosomes

Gosavi, Ujwala A. (UCONN health)*; Srivastava, Ankita (UCONN health); Gunzl, Arthur (UCONN health)*

The cyclin-dependent kinase CRK9 (CDC2-related kinase 9) was the first trypanosome CDK that exhibited control over gene expression. CRK9 silencing was lethal in cultured trypanosomes, causing a block of spliced leader (SL) trans-splicing of nuclear pre-mRNA and dephosphorylation of RPB1, the largest subunit of RNA polymerase II.Towards identifying CRK9 substrates and characterizing regulatory signaling pathways of gene expression, we generated cell lines that exclusively express C-terminal PTP-tagged, ATP analog-sensitive (AS) CRK9 in which the gatekeeper residue M438 was substituted with glycine (CRK9AS1-PTP) or alanine (CRK9AS2-PTP). These cells exhibited similar CRK9 protein expression but showed mild growth defects compared to cells expressing CRK9 WT-PTP with wild-type gatekeeper. Treatment of these cell lines with three different bulky, N6-enlarged ATP analogs, revealed within 48 hours that one of the drugs, 1-NM-PP1, significantly inhibited culture growth of CRK9AS1-PTP-expressing cells, causing them to round up, a phenotype characteristic for CRK9-silenced cells. The compound was effective at an EC50 value of ~1.5 µM for these cells whereas it was tenfold higher for CRK9WT-PTP-expressing cells, indicating that the inhibitor effectively blocked the activity of CRK9AS1-PTP. When treated with 10 µM of 1-NM-PP1, block of trans and cis (intron removal) splicing became apparent after 5 and 60 minutes, respectively, whereas it took ~6 hours before RPB1 dephosphorylation was detectable. These results strongly indicate that the spliceosome requires continuous input from CRK9 to remain active, strengthening the notion that CRK9 is a valid drug target against trypanosomatid parasites. Since we discovered that purified CRK9AS1-PTP but not CRK9WT-PTP accepts “bulky” ATP?S for thio-phosphorylation, we are in the process to employ this reaction for the identification of CRK9 substrates.

126: Analyzing the basic function of the positionally conserved PAP1 intron in Trypanosoma brucei

Srivastava, Ankita (Department of Genetics and Genome Sciences, UConn Health, Farmington, CT); *O'Connor, Zachary (UCONN health); Gunzl, Arthur (UCONN health)*

Kinetoplastids include trypanosomes and leishmanias (trypanosomatids), and the free-living Bodo saltans. The latter diverged from the trypanosomatid lineage ~500 million years ago before the emergence of insects, amphibians and reptiles. As our analysis indicates, B. saltans and trypanosomatids for which genome data are available harbor an intron that disrupts the codon of a conserved amino acid of poly(A) polymerase 1 (PAP1; Tb927.3.3160) and an intron disrupting the codon of an identical amino acid of RNA helicase DBP2B (Tb927.8.1510). Moreover, independent transcriptome analyses revealed that these are the only two pre-mRNA introns in Trypanosoma brucei. Since ancestors to extant eukaryotes likely had intron-rich genomes, it appears that T. brucei has eliminated all but these two introns. Given their positional conservation among kinetoplastids and their retention in the T. brucei genome, we hypothesize that the PAP1/DBP2B introns serve fundamentally important functions. PAP1 is essential for snoRNA biogenesis and trypanosome viability. To investigate effects of intron deletion, we functionally fused PAP1 at its N-terminus with the PTP tag in procyclic trypanosomes, allowing us to specifically monitor expression from the manipulated PTP-PAP1 allele. By generating PTP-PAP1Intron/No Intron::PAP1 and PTP-PAP1Intron/No Intron::HYG cell lines, we discovered that intron removal increased PAP1 expression more than 2.5 fold at RNA and protein levels, revealing a negative role for the intron in PAP1 expression. Although PTP-PAP1No Intron::HYG cells had lower overall PAP1 mRNA abundance than PTP-PAP1No Intron::PAP1 cells, they proliferated distinctly slower, suggesting that the intron not merely reduced PAP1 expression but regulated the relatively short-lived PAP1 mRNA (t½ ~21 min), f.ex. during the cell cycle. We will use conditional PAP1No Intron overexpression to study defects of intron elimination.

127: How is the Glideosome Associated Connector driving parasite motility?

Dos Santos Pacheco, Nicolas (University of Geneva); *Vadas, Oscar (University of Geneva); Jacot, Damien (University of Geneva); Tosetti, Nicolò (University of Geneva); Han, Huijong (University of Oulu); Kursula, Inari (University of Oulu - University of Bergen); Soldati-Favre, Dominique (University of Geneva)*

In Toxoplasma gondii, gliding motility is a prerequisite for host cell invasion, parasite egress and dissemination. This process relies on the rearward translocation of apically discharged transmembrane adhesins (MICs) by the glideosome, a phylum-specific actomyosin system. The MICs binds extracellular matrix and are connected to the parasite F-actin by the glideosome associated connector (GAC). GAC is an essential modular protein with an N-terminal domain binding to F-actin and a C-terminal PH domain binding to phosphatidic acid (PA). PA is transiently produced at the parasite plasma membrane during microneme secretion and possibly contributes to GAC affinity to the inner leaflet of the plasma membrane, facilitating its interaction with the cytoplasmic tails of MICs. Previous studies highlighted conserved amino acid within MIC2 tail that are required for parasite survival. Using Surface Plasmon Resonance experiments we demonstrated that while the wild-type MIC2 tail binds to GAC, the mutant MIC2WE/AA failed. Insertion of the mutation in the endogenous MIC2 locus via CRISPR/Cas9 resulted with a decrease in parasite gliding motility and a reduced gliding speed while attachment and invasion were not affected. These data support the model where adhesins have a dual role during motility, first in surface attachment with the ectodomain and then in force transmission via the cytoplasmic tail. To gain further insight into the molecular interactions between GAC, PA and MIC2, we are performing GAC-lipid co-sedimentation experiments using PA containing liposomes decorated with MIC2 tails. Additionally, an Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) approach will be implemented to identify interacting regions for each partner. Finally, we are in the process of obtaining a high-resolution structure of GAC by cryo-electron microscopy.

128: A novel lipid-binding protein mediates rhoptry discharge and invasion in Plasmodium falciparum and Toxoplasma gondii parasites

Lebrun, Maryse (CNRS-Université Montpellier 2); *Suarez, Catherine (UMR5235 CNRS Université Montpellier); Lentini, Gaelle (UMR5235 CNRS Université Montpellier); Maynadier, Marjorie (UMR5235 CNRS Université Montpellier); Aquilini, Eleonora (UMR5235 CNRS Université Montpellier); Ramaswamy, Raghavendran (University of Victoria); Berry-Sterkers, Laurence (UMR5235 CNRS Université Montpellier); Cipriano, Michael (Univeristy of Georgia); Chen, Alan (University of California, Los Angeles); Bradley, Peter (University of California, Los Angeles); Striepen, Boris (School of Veterinary Medicine, University of Pennsylvania); Boulanger, Martin (University of Victoria)*

The phylum Apicomplexa comprises obligate intracellular pathogens responsible for important human diseases including malaria (Plasmodium spp.), cryptosporidiosis (Cryptosporidium) and toxoplasmosis (Toxoplasma gondii). It is defined by the presence of an apical complex and two types of secretory organelles (micronemes and rhoptries), which sequentially release their contents during invasion. Initially, the micronemes are secreted in a calcium-depending fashion, followed by rhoptry exocytosis. During invasion, rhoptries inject an array of invasion and virulence factors into the cytoplasm of the host cell. Analogous to bacterial secretion systems, rhoptry secretion is essential for the intracellular lifestyle of these divergent eukaryotes, but the molecular mechanism of rhoptry discharge remains unknown. Here we identified a set of novel parasite specific proteins, termed rhoptry apical surface proteins (RASP) that cap the extremity of the rhoptry at its intersection with the plasma membrane. Depletion of RASP2 results in loss of rhoptry secretion and completely blocks parasite invasion and therefore parasite proliferation in both Toxoplasma and Plasmodium. Recombinant RASP2 binds charged lipids and likely contributes to assembling the machinery that docks/primes the rhoptry to the plasma membrane prior to fusion. This study provides mechanistic insight into a parasite specific exocytic pathway, essential for the establishment of infection.

129: Functional mapping of the ap2-g promoter in Plasmodium falciparum

Basson, Travis (Swiss TPH, University of Basel); *Voss, Till S. (Swiss TPH, University of Basel)*

The generation of non-proliferating blood stage forms of Plasmodium falciparum known as gametocytes is a prerequisite for the transmission of malaria between humans via the mosquito vector. Gametocytes are produced from a small proportion of replicating intra-erythrocytic parasites via a process known as sexual commitment. The transcription factor AP2-G is the master regulator of sexual commitment. The ap2-g locus is epigenetically silenced by heterochromatin protein 1 (HP1), which antagonizes sexual conversion in proliferating parasites. The activation of this locus is triggered by the upstream regulator gametocyte development protein 1 and responsive to deprivation of the host-derived serum lipid, lysophosphatidylcholine (LysoPC). Parasites expressing AP2-G make the cell fate decision to exit asexual replication and undergo gametocyte differentiation. Silencer elements, potentially located within the upstream promoter region of ap2-g, are presumably responsible for establishing silenced chromatin at the locus in order to maintain asexual replication cycles. Here, we used CRISPR/Cas9-based gene editing to insert a reporter cassette where the ap2-g promoter controls expression of the drug-selectable marker blasticidin deaminase fused to the green fluorescent protein (BSD-GFP), as well as Nano-Luciferase (N-LUC), into the dispensable cg6 locus. In this context, the readouts of the BSD-GFP fusion and N-LUC reporters allow us to investigate ap2-g promoter silencing at the single cell and population levels. Together, we aim to use this system for the identification of putative cis-acting elements and regulatory factors implicated in the epigenetic regulation of the ap2-g locus and sexual commitment.

130: Charting the Basis for T. gondii Virulence Traits Through Adaptive Laboratory Evolution (ALE)

Primo, Vincent (Boston College); *Farrell, Andrew (University of Utah School of Medicine); Rezvani, Yasaman (University of Massachusetts at Boston); Vajdi, Amir (University of Massachusetts at Boston); Marth, Gabor (University of Utah School of Medicine); Zarringhalam, Kourosh (University of Massachusetts at Boston); Gubbels, Marc-Jan (Boston College)*

Having a genetic difference of only 0.002%, RH and GT1 (both of Type I) show remarkable phenotypic differences in vitro, including plaque size and extracellular survival. Since RH has been in in vitro for ~40 years, lab adaptation is likely the root of these differences. Replaying the evolutionary changes that occur throughout Adaptive Laboratory Evolution (ALE) by serial passaging of T. gondii may help to identify alleles, expression patterns, and epistatic relationships associated with such host-independent virulence traits. Here we have subjected non-lab-adapted GT1 strain tachyzoites to ALE for >200 passages (~550 generations, ~2 years) and observe the evolution of several phenotypes over time. Upon serial passaging of GT1, we observed a steady increase in plaque size (2.85 fold), an increase in reinvasion efficiency (2.63 fold), and an increase in extracellular survival (2.15 fold). Comparative genomics of 4 clones using Whole Genome Shotgun Sequencing during ALE revealed an accumulation of mutations occurring relatively early during ALE. However, many of these mutations occur in non-coding regions and are unlikely the root of phenotypic changes. Therefore we investigated changes in mRNA profiles through mRNA-seq. This identified many differentially regulated genes over time, correlating with the observed change in phenotypes. To better pinpoint expression patterns specific to extracellular survival, mRNA-seq was performed on 6-hour-extracellular GT1 parasites, again over the course of ALE. Thousands of genes were identified as being differentially expressed upon prolonged extracellular conditions, including many AP2 and SRS genes. In conclusion, having identified phenotypic, genomic, and transcriptomic changes, ALE of the non-lab-adapted GT1 strain is a promising approach for identifying the basis for host-independent virulence traits in the tachyzoite.

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

***Klingbeil, Michele M. (University of Massachusetts, Amherst)**; Rocha-Granados, Maria C. (University of Massachusetts Amherst); Dodard, Garvin A. (Brown University); Gunzl, Arthur (University of Connecticut Health Center)*

Replication, transcription and chromatin remodeling are coordinated to ensure accurate duplication of genetic and epigenetic features in all eukaryotes. Although recent studies in Trypanosoma brucei indicate functional links among DNA replication, transcription and antigenic variation, mechanisms that coordinate these essential processes remain largely unknown. T. brucei and related parasites display unusual properties in regard to DNA replication including a highly divergent Origin Replication Complex, and few origins of replication compared to model eukaryotes. Thus far, studies of protein dynamics at replication forks have been mainly confined to gene-by-gene investigations with few opportunities to study the entire machinery in a temporal fashion. Therefore, we adapted technology called iPOND (isolation of proteins on nascent DNA) and coupled it with mass spectrometric analysis as an unbiased tool to identify proteins associated with DNA replication forks of T. brucei. Several key optimizations were necessary to adapt the technology successfully to trypanosomes. Proteomic analyses revealed that, in addition to the core replication machinery (7.89-fold enrichment via GO analysis), proteins associated with transcription (7.21), chromatin assembly (8.93) and DNA repair (6.49) were enriched at unperturbed DNA replication forks. Additionally, 98 proteins of unknown function were enriched which may represent trypanosome-specific factors or divergent homologs of known replication proteins and, thus, may constitute potential new drug targets. To assess replication defects, we silenced replication factor C, subunit 5 (RFC5) and analyzed EdU incorporation through high-throughput microscopy. RFC5 depletion resulted in loss of fitness, and a significant reduction in EdU incorporation indicating an essential replication function. Thus, we have set the stage for a comprehensive analysis of the unusual trypanosomatid replication machinery under unperturbed and damage induced conditions.

132: Mobile Games: A Novel Instructional Tool to Teach Invasion of Red Blood Cells by Plasmodium falciparum

Comunale, Mary Ann (Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia PA)*; Harvey, John (Game Design and Production, Westphal College of Media Arts & Design, Drexel University, Philadelphia, PA); Vaidya, Akhil (Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia PA); Burns, Jim (Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia PA); Bergman, Lawrence (Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia PA); Wigdahl, Brian (Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia PA); Urdanta-Hartmann, Sandra (Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia PA)*

In the life sciences, lectures and extensive reading assignments continue to be the standard teaching practice. Interactive digital media platforms offer new and exciting opportunities for educators to provide active and engaging learning strategies that motivate and increase visual learning attributes of their courses. Current students have grown up in a digital age, and are accustomed to being connected to online to resources that provide immediate information. The popularity of smartphones has added the ability to access that information anytime anywhere. The implications on learning have been substantial. Digital games, and mobile games in particular, are another product of this digital era that has enjoyed immense popularity with people of all ages. Studies have shown that games can provide an engaging and motivational learning experience. We aim to utilize smartphone mobility in conjunction with the motivational and engaging aspects of game based learning to develop convenient and exciting educational mobile games that teach topics in infectious disease. Our first iOS and Android mobile game CD4 Hunter™ has proven to be an effective tool to teach about how HIV infects CD4+ T cells to higher education students. We are now developing a mobile game about the molecular mechanisms by which Plasmodium falciparum merozoites invade red blood cells. Our method for designing and developing this game, educational content, and suggestions for implementation in higher education courses are presented. Also, participants will be able to play the game, scheduled to be launched in iTunes and Google Play app stores in September 2018.Key Words/Phrases: Mobile Games, Game Based Learning (GBL), malaria, merozoites, e-learning, higher education.

133: Identification of Plasmodium intrinsic factors influencing gametocytes infectivity to Anopheles mosquitoes in the field

Ouologuem, Dinkorma (MEDRU / MRTC/ FAPH/ USTTB); *Kone, Aminatou (MEDRU / MRTC/ FAPH/ USTTB); Dara, Antoine (MEDRU / MRTC/ FAPH/ USTTB); Diallo, Nouhoum (MEDRU / MRTC/ FAPH/ USTTB); Dembele, Laurent (MEDRU / MRTC/ FAPH/ USTTB); Ballo I., Fatoumata (MEDRU / MRTC/ FAPH/ USTTB); Sangare, Cheick Oumar (MEDRU / MRTC/ FAPH/ USTTB); Sangare, Boubou (MEDRU / MRTC/ FAPH/ USTTB); Haidara, Aboubecrin (MEDRU / MRTC/ FAPH/ USTTB); Dembele, Demba (MEDRU / MRTC/ FAPH/ USTTB); Traore, Aliou (MEDRU / MRTC/ FAPH/ USTTB); Doumbo, Ogobara K. (MRTC/ FMPOS/ USTTB); Djimde, Abdoulaye (MEDRU / MRTC/ FAPH/ USTTB)*

Sustainable malaria prevention and control measures have permitted a significant decrease in the disease burden in many endemic countries. However, the global agenda for malaria elimination and eradication may not be achieved without the development of transmission-blocking interventions.
Malaria transmission to the mosquito relies on gametocytes infectivity, which ensures both the continuation of the parasite life cycle and the spread of resistant parasites. Identification of biomarkers associated with gametocyte infectivity can help in the development of new therapeutic strategies. This study aimed to identify host and parasite biomarkers related to gametocyte infectivity in the field.From October to December 2017, a cross-sectional study was conducted in Faladje to enroll asymptomatic Plasmodium ssp. gametocyte carriers. Gametocyte carriage was assessed by light microscopy. Venous blood was collected for gametocytes purification, plasma collection and direct membrane feeding assay (DMFA) with both whole blood and RBC supplemented with AB serum. Mosquito's oocyte positivity was determined day-8 post feeding. Protein microarrays covering 250 P. falciparum antigens were probed with plasma from volunteers. Gametocyte's RNA and DNA were extracted for RNA and DNA sequencing.70 volunteers were enrolled. For the 68 malaria positive samples used for DMFA, mosquito's infectivity rate was

28% (n=19). The number of oocyst positive mosquito and oocyst load per mosquito were significantly higher in RBC+AB serum DMFA for 73.7% (n= 14) of the infectious sample, suggesting negative effect of serum containing factors on parasite lifecycle within the mosquito. Ab levels to 7 antigens were significantly higher in plasma from non-infectious gametocyte blood meals compared to those who were infectious. Candidate antigens included a Plasmodium Deoxyhypusine hydroxylase, four putative proteins highly expressed in gametocytes, ookinetes and/or sporozoites, and an ATG autophagic pathway associated protein. RNA sequencing result from infectious and non-infectious gametocyte will be compared to identify genes with significant expression difference between the two phenotypes.Dissecting factors influencing gametocytes infectivity will highlight biomarkers associated with malaria transmission and provide potential targets for transmission blocking strategies and new tools for malaria transmission screening.

134: Antimalarial drug exposure triggers the formation of cytosolic hemozoin compartments in Plasmodium falciparum parasites

Maleki, Sharareh (McGill University); *Rohrbach, Petra (Institute of Parasitology, McGill University)*

Chloroquine (CQ) treatment failure in Plasmodium falciparum parasites has been documented for decades and many studies have attempted to resolve the antimalarial mechanism of action of CQ but the pharmacological explanation of this phenotype is not fully understood. Current concepts attribute CQ resistance to reduced accumulation of the drug at a given external CQ concentration ([CQ]ex) in resistant compared to sensitive parasites. The implication of this explanation is that the mechanisms of CQ-induced toxicity in resistant and sensitive strains are similar once lethal internal concentrations have been reached. We have reported differences in killing kinetics and cell biological consequences in CQS and CQR parasites after exposure to equipotent [CQ]ex, determined based on IC50 values in assays quantifying growth inhibition.

Additionally, we found the appearance of dark cytosolic structures (DCS) containing hemozoin (Hz) in the cytosol of CQS but not CQR parasites treated with CQ. Interestingly, the DCS were not found in CQ treated CQS parasites in the presence of verapamil. We are now looking at additional antimalarial drugs (quinine, mefloquine and amodiaquine) to determine if these drugs also cause the parasite to form DCS in the cytosol of drug-sensitive, and not drug-resistant, parasites. To date, our findings suggest that sensitive parasites form DCS on exposure to quinine and mefloquine, and resistance parasites require much higher levels of drug before DCS are formed. We conclude that the formation of DCS is a form of parasites response to antimalarials and this phenomenon could be a part of events which leads to the death of the parasite.

135: Comparative Genomics and Network Modeling of Parasites

Carey, Maureen (University of Virginia); *Stolarczyk, Michal (University of Virginia); Untariou, Ana (University of Virginia); Medlock, Gregory (University of Virginia); Guler, Jennifer (University of Virginia); Papin, Jason (University of Virginia)*

Experimentally tractable model organisms are used to interrogate disease and parasite phenotypes, but characterization of functional differences between parasite species is limited to post hoc and single target studies. Each parasite genome encodes unique enzymes; however, it is unclear whether these differences arise from divergent metabolism or incomplete genome annotation. To address this challenge, we generated metabolic reconstructions from 160 parasite genomes, supplemented with experimental data and by inferring function to complete or connect metabolic pathways. With these 160 metabolic reconstructions, representing 38 genera and 111 species, we compare metabolic capacity, gene essentiality, and pathway utilization. Nearly 46% of models contain at least one unique reaction not in any other model.

Unsurprisingly, the large genome of Chromera velia has the most unique reactions (35), but the smallest genome (Plasmodium billcollinsi) also has six unique reactions. While the size of model is correlated with the genome size, even small models contain unique reactions.to validate our networks, we compare in silico predictions with experimental results. Here, we focus on two Plasmodium species (falciparum and berghei) in which genome-scale essentiality screens have been conducted. We compare experimental data with in silico predictions, and by identifying inconsistencies between the data and predictions, we generate targeted experimental hypotheses for improving genome annotation. Moving forward, by exploring metabolic differences between organisms, we can generate testable hypotheses about species specific functions. For example, P. falciparum grows in anucleated host erythrocytes, whereas P. berghei prefers nucleated reticulocytes. We find P. falciparum has a reduced demand for host purines when compared to P. berghei, perhaps explaining this host cell preference. By performing these analyses with all parasite models, we can also identify metabolic discrepancies and commonalities between genera and species, facilitating comparison of experimental findings and optimizing model organism selection.

136: TgPL3 is a microtubule associated virulence factor with patatin-like phospholipase A2 and lipoxygenase activity

Wilson, Sarah (University of Wisconsin Madison)*; Koch, Lindsey (University of Wisconsin Madison); Morrissette, Naomi (University of California Irvine); Knoll, Laura (University of Wisconsin Madison)*

Toxoplasma gondii elicits a complex immune response throughout the course of infection. Phospholipase A2 (PLA2) enzymes cleave the sn-2 bond of phospholipids to release polyunsaturated fatty acids (PUFAs), which can be modified to regulate both lipid metabolism and inflammation regulation.
Lipoxygenases (LOX) are a family of iron-binding enzymes that catalyze the dioxygenation of those PUFAs to produce lipid mediators of inflammation. While humans have multiple PLA2 and LOX enzymes to produce eicosanoids that regulate immune response, T. gondii also has PLA2 and LOX activity.
Analysis of the T. gondii genome shows six proteins with patatin-like PLA2 domains (TgME49_254420, 231370, 232600, 273730, 212130, and 305140) and four potential LOX enzymes (TgME49_315970, 202970, 239580, and 305140).TgME49_305140 (TgPL3) is a 277 kDa protein with combined patatin-like PLA2, LOX and microtubule binding domains. Extraction of parasites with deoxycholate shows that TgPL3 binds to microtubules and is localized to the tips of subpellicular microtubules immediately below the apical polar ring. Staining of extracted parasites suggests that it may also localize to extruded conoid fibers. Overexpression of TgPL3 in T. gondii shows increased PLA2 activity in the lysates. Purified TgPL3 LOX domain from E. coli shows significant LOX activity. To our knowledge, TgPL3 represents the first protein to contain combined PLA2 and LOX activity. Mice infected with a TgPL3 mutant survive more often to chronic infection, have fewer brain cysts and have higher levels of pro-inflammatory cytokines. ?TgPL3 parasites show an invasion defect in tissue culture. Characterizing TgPL3 will elucidate its dual-function in invasion and manipulating the innate immune response that controls tachyzoite growth.

137: Small volume, cryopreserved Plasmodium vivax isolates for RNAseq studies via the SmartSeq2 platform

Rangel, Gabriel (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA)*; Clark, Martha (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA); Goldberg, Jon (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA); Kanjee, Usheer (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA); Ferreira, Marcelo (Department of Parasitology, Institute of Biomedical Science, University of Sao Paulo, Sao Paulo, Brazil); Nusbaum, Chad (Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA); Neafsey, Daniel (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA; Broad Institute of Harvard and MI); Duraisingh, Manoj (Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA; Broad Institute of Harvard and MI)*

Plasmodium vivax is the most geographically widespread human malaria parasite, putting approximately one-third of the global population at risk of infection, resulting in up to 20 million clinical cases in 2016. Although, P. vivax research is currently limited by the lack of a continuous in vitro culture system, recent work from our group has optimized the use of small-volume cryopreserved P. vivax isolates from human donors for the short-term culture by improving parasite enrichment techniques and identifying IMDM as a media formulation that greatly improves parasite survival. Pairing our improved culture system with low-input SmartSeq2 RNAseq technology, we probed the transcriptional signature of ex vivo P. vivax parasites over the course of intraerythrocytic maturation, generating data with robust coverage of the P. vivax transcriptome. We isolated transcripts from sexual stage P. vivax gametocytes, yielding an unbiased, RNAseq-based gametocyte transcriptome. Furthermore, we describe the general transcriptional differences between multiple isolates from different patients, and more specifically the differential expression of P. vivax invasion ligands at schizogony, as well as the effect of culture media on parasite transcriptional signatures. These data demonstrate the feasibility of unbiased transcriptional studies from small volume cryopreserved P. vivax isolates.

138: A parasite phosphorylation-ubiquitination axis licenses egress of Plasmodium falciparum from host erythrocytes

Paul, Aditya S. (Harvard T.H. Chan School of Public Health)*; Paulo, Joao A.; Goldberg, Jon; Elsworth, Brendan; Kosber, Aziz L.; Gygi, Steven P.; Duraisingh, Manoj T.*

There is limited understanding of the key transitions of the cell cycle of malaria parasites that drive replication within an erythrocyte and dissemination to new host cells. To probe the parasite cell cycle, we generated transgenic Plasmodium falciparum for conditional knockdown of the parasite homolog of the Protein Phosphatase 1 (PP1), a highly conserved enzyme for signal transduction used for mitotic progression in diverse eukaryotes. We found that Pf-PP1 is essential to blood-stage parasites, with induced protein degradation eliciting a complete block in proliferation. Studies of the stage-specificity of the knockdown-related phenotype show that Pf-PP1 is critical for intracellular parasite replication: (i) knockdown at an immature stage delays entry into the mitosis; (ii) knockdown in mitotic parasites reduces replication of nuclei by as much as ~2-3-fold; and (iii) late in mitosis when DNA synthesis is nearly complete, knockdown blocks further developmental progression and transition to egress. Chemical-genetic-based studies to test for synthetic, functional interactions provide support for Pf-PP1 in mitotic signaling, and proteomic analysis of Pf-PP1 knockdown suggests importance for transition from replication to egress. A phospho-proteomic analysis of Pf-PP1 knockdown in late mitotic parasites shows an increase in phosphorylation of a parasite-specific HECT E3 ubiquitin-ligase, raising the possibility of regulation of function. Follow-up chemical biology-based screening shows that treatment of P. falciparum with heclin, a small molecule inhibitor of HECT-mediated ubiquitination, phenocopies knockdown of Pf-PP1; and parasites depleted of the phosphatase are strongly sensitized to heclin (up to ~10x reduction in IC50). We propose that Plasmodium phosphorylation-based signal tranduction converges with ubiquitination to regulate mitotic exit and differentiation of malaria parasites into invasive forms competent for host cell egress.

139: Investigating the Plasmodium epitranscriptome as a translational control mechanism

Sinha, Ameya*; Chee Sheng, Ng; Nah, Qianhui; Dedon, Peter; Preiser, Peter*

Enzyme-catalyzed modifications of ribonucleic acids (RNA) occurs at a number of base and sugar positions and has the potential to influence specific anticodon-codon interactions and regulate translation. Recent evidence provides strong support for the idea that cells use changes in the nucleotide modifications in transfer RNA (tRNA) to dynamically regulate gene expression in response to stress. Using a LC-MS/MS-based platform, we have characterized the full spectrum of modified ribonucleosides on the transfer RNA (tRNA) in *P. falciparum*. We have then quantified tRNA modification levels in the parasites that were exposed to oxidative stressors like hydrogen peroxide, sodium hypochlorite and a range of anti-malarials like artemisinin, chloroquine, and pyrimethamine. Differences in the tRNA modifications observed in response to the range of stressors would suggest that regulation of modifications enables the parasite to activate alternative stress response pathways. The work presented here holds potential for several avenues of new discovery. For one, unique patterns of tRNA modification reprograming can distinguish mechanistically distinct damaging agents which supports the idea the tRNA modification patterns can be used as biomarkers of exposure. Besides these stress-specific patterns of tRNA modification changes are linked to selective translation of codon-biased mRNAs for stress response proteins in other organisms. In order to address this hypothesis in *Plasmodium* we have decided to carry out a proteomics experiment to determine if the parasite proteins that are upregulated show a marked codon bias which can be tied up with the tRNA modification data. All of these findings together provide new insights into the role of translational control mechanisms involving tRNA modifications in the development and pathogenesis of malaria parasites.

140: Disruption of the de novo heme biosynthesis pathway leads to severe defects of growth and acute virulence in Toxoplasma gondii

***Dou, Zhicheng (Clemson University)**; Floyd, Katherine (Clemson University); Bergmann, Amy (ClemsoN University); Dameron, Carly (Clemson University)*of Toxoplasma infection.

Therefore, the deletion of TgCPOX blocks the utilization of host cytosolic heme intermediates. We also functionally complemented TgCPOXin the yeast mutant (?hem13) which lacks the ortholog of TgCPOX, proving TgCPOX is a functional enzyme. We also successfully ablated the TgCPOXgene by homologous recombination. The resulting TgCPOX-null mutants exhibited approximately 70% reduction of total heme, compared to WT parasites, and showed smaller plaques and slower replication than WT parasites. More strikingly, the mutant parasites displayed severely decreased virulence in a murine model, suggesting that the de novoheme biosynthesis pathway plays a key role in the pathogenesis of toxoplasmosis. The addition of heme to the growth medium did not rescue the defects in plaque formation, which excludes the possibility that Toxoplasma can access extracellular free heme. The viability of the ? cpoxindicates that the parasites possess a non-canonical bypass pathway for heme biosynthesis. The preliminary results from RNA-Seq analysis identified three radical S-adenosylmethionine enzymes become up-regulated in ?cpox. These enzyme(s) could serve as oxygen-independent coproporphyrinogen III oxidase to supplement heme production in Toxoplasma. Collectively, our findings will shed light on the development of novel strategies to block heme production and acquisition to benefit clinical management

Toxoplasma gondii is an Apicomplexan parasite that may cause severe infectious disease in humans, with the ability to use foodborne, zoonotic, and congenital routes of transmission. Toxoplasma de novo biosynthesizes and also scavenges small nutrient molecules from host cells, such some amino acids, nucleotides, and simple lipids, to support its intracellular growth. Heme is an essential nutrient in all organisms, serving as a prosthetic group conjugated to many proteins for many fundamental subcellular activities. Toxoplasma encodes all the enzymes in the de novo heme biosynthesis pathway in its genome. The evidence of mass spectrometry and endogenous gene tagging establish their expression during acute infection. In this study, we aimed to the gene coding coproporphyrinogen III oxidase enzyme (TgCPOX) that catalyzes the antepenultimate reaction within the pathway in Toxoplasma. Among the entire pathway in Toxoplasma, this step is the only reaction occurring in the cytosol. However, mammals generate the products of all reactions upstream the step CPOX catalyzes in the cytosol.

141: Single Oral Dose Cethromycin Cures P. berghei Liver Stage Malaria Initiated By Mosquito Bites

Sullivan, David J*. (Johns Hopkins Bloomberg School of Public Health); Kennedy, Grace (Johns Hopkins Bloomberg School of Public Health); Evans, Rachel (Johns Hopkins Bloomberg School of Public Health); Poti, Kristin (Johns Hopkins Bloomberg School of Public Health); Bobb, Bryce (Johns Hopkins Bloomberg School of Public Health); Kaludov, Nick (Aliquantum Rx)*

We investigated the liver stage pharmacokinetics and pharmacodynamics of cethromycin, a hybrid of quinoline and erythromycin, in the P. berghei liver stage model initiated by mosquito bites. Here we demonstrate complete cure of P. berghei infection by single oral dose of 60 mg/kg in mice which is equivalent to the 5 mg/kg human dose of 300 mg a day used in bacterial pneumonia studies. Both quinoline and erythromycin alone at 120 mg/kg for two doses as well as control mice resulted in patent blood stage parasitemia in all mice. Cethromycin at 30 mg/kg was also curative as well as single oral 60 mg/kg given before mosquito infection.
Immunofluorescence imaging of cethromycin treated, in vitro hepatocyte infected cells shows complete ablation of the apicoplast. Regarding blood stage activity, cethromycin at 60 mg/kg daily for 7 days cured in a high parasitemic P. berghei mouse model. While mosquito membrane feeding of P. falciparum gametocytes incubated with 20 microM cethromycin demonstrated no decrease in oocyst numbers, oral dosing in mice showed significant decrease in both prevalence and mean number of oocysts suggesting an active metabolite similar to primaquine. Liver pharmacokinetic studies in mice show peak milliMolar drug levels in the liver with 20 hour sustained levels above 10 microMolar for nearly 24 hours. The liver concentrations were higher than the lung and about 100 times that of blood. Cethromycin has been evaluated for efficacy against bacterial pneumonia in more than 5,000 patients with good safety profiles. Cethromycin has potential for rapid clinical development for casual malaria prophylaxis and possibly radical cure of dormant liver P. vivax or P. ovale.

142: Loss of a deubiquitinase, TgOTUD5 delays cell cycle progression and breakdown of maternal remnants in Toxoplasma gondii.

Dhara, Animesh *(University of Kentucky); Murphy, Robert (University of Kentukcy College of Medicine); Sinai, Anthony P. (University of Kentukcy College of Medicine)*

The functions of Ovarian TUmor (OTU) domain containing deubiquitinases (DUBs) are beginning to be explored in Apicomplexa. Our recent work (mBio.8:e01846-17) had shown that TgOTUD3A, is critically important for selecting the cell cycle strategy in T. gondii. In this study we functionally characterize another cell cycle regulated DUB, TgOTUD5 (TGGT1_243510). C-terminal HA-taggedTgOTUD5 revealed a cytoplasmic localization with cell cycle dependent protein expression which is the lowest at M/C boundary, increasing with the progression of cytokinesis and peaking in G1 after the emergence of daughter progeny. In light of a significant fitness defect in a recent CRISPR screen, we established a conditional line with a HA-tagged TgOTUD5 under the control of a TET-regulated minimal SAG4 promoter.

Despite abolishing normal transcriptional regulation using the constitutive TET-SAG4 promoter, TgOTUD5-HA followed the endogenous expression pattern suggesting regulation by proteasomal turnover as evident following proteasome inhibition. Under conditional knock-down conditions, TgOTUD5 levels are below the detection limit within 24 h resulting in the parasites exhibiting a delay in cell cycle progression accompanied by defective breakdown of maternal remnants following 2 days of ATc treatment. This results in a major reduction in both plaque size and number. An E. coli expressed recombinant enzyme showed almost no activity against synthetic poly-Ub peptides while exhibiting a specifc high affinity interaction with the linear Ub chains. This presents the possibility that TgOTUD5 could act as scaffold in a ubiquitin complex. The orthologous human enzyme expressed in insect cells showed activity as it requires a post-translational modification. We are currently attempting the insect cell expression of TgOTUD5 to understand its biochemical function in the context of parasite cell cycle.

143: The glucan phosphatase, TgLaforin, regulates amylopectin metabolism in both T. gondii tachyzoites and bradyzoites.

Murphy, Robert D. *(University of Kentucky); Dhara, Animesh (University of Kentucky); Watts, Elizabeth (University of Georgia); Brizzee, Corey (University of Kentucky); Sinai, Anthony (University of Kentucky); Gentry, Matthew (University of Kentucky)*

A distinguishing characteristic of cyst forming Apicomplexa is the accumulation of a starch-like glucose polymer known as amylopectin granules (AGs) within bradyzoites. The AG levels within encysted T. gondii bradyzoites are highly variable, suggesting their accumulation and depletion is under homeostatic control. In light of our findings that encysted bradyzoites retain replicative capacity, we propose that AGs serve as a glucose cache for energy needs.

Accessing glucose within insoluble starch, a cycle of phosphorylation/dephosphorylation of the starch surface is required. Importantly, the T. gondii genome encodes the activities needed for starch turnover. These include a glucan dikinase ("TgGWD": TgME49_214260) and a glucan phosphatase ("TgLaforin": TgME49_205290), in addition to multiple amylases. Bioinformatic analyses indicate that TgLaforin encodes a unique carbohydrate binding module and a dual specificity phosphatase domain.

Expression of active recombinant TgLaforin necessitated expression of a codon optimized synthetic gene in insect cells. Enzymatic activity assays confirm that recombinant TgLaforin is an active glucan phosphatase. Moreover, we have demonstrated that a CRISPR-Cas9 knockout of TgLaforin in tachyzoites results in a slow developing starch accumulation phenotype that results in growth inhibition, taking roughly 3 weeks to manifest. This accumulation of AGs in tachyzoites promotes compensatory changes resulting in the selection for variants that no longer accumulate AGs, a phenotype similar to what we observed in Cyanidioschyzon merolae, a red algae with close evolutionary ties to T. gondii. We are now investigating the possibility that this population is refractory to stress induced stage conversion in cultured cells.

These findings suggest that AG metabolism may play roles not only in bradyzoites, but also within tachyzoites and their capacity to stage convert.

144: Hemozoin in isogenic drug-resistant P. falciparum is smaller in size

Sayeed, Abeer *(Johns Hopkins Bloomberg School of Public Health)*

Heme metabolism is a central high throughput pathway for Plasmodium. The quinolones target heme by inactivation to form heme crystals which removes the reactive iron from reduction-oxidation chemistry. In contrast, the artemisinins are activated by reduced heme or iron to generate radicals which damages nearby proteins and enzymes. Artemisinin ring-stage resistant P. falciparum parasites display a delayed clearance phenotype associated with genetic selection for Kelch 13 mutations with the C580Y predominate. Here we investigated the hemozoin morphology in the Cambodia Wild Type strain (CamWT) and its isogenic C580Y cloned isolate. Mean crystal width was 131nm and length was 556nm in CamWt compared to the smaller 113nm width and 532nm length of C580Y. Inspection of the chloroquine sensitive 106/1 and the chloroquine resistant FCB showed mean hemozoin width of 112nm in 106/1 with length of 476nm while the FCB hemozoin was smaller in both dimensions-width 98nm and length 412nm. Likewise, in chloroquine resistant NYU-2 P. berghei, the hemozoin width was 25nm smaller while length was 40nm smaller than chloroquine-sensitive hemozoin. In non-isogenic drug-resistant and drug sensitive P. falciparum isolates the range was diverse in the hemozoin dimensions. Single point mutations are sufficient to produce smaller hemozoin crystals. Future work will address correlation of cell cycle lengths with hemozoin dimensions in isogenic strains and how smaller hemozoin production is a consequence of parasite stress response.

145: Rapid and specific drug-induced transcriptional responses to common antimalarials in asexual P. falciparum

Painter, Heather *(Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803, USA); Llinás, Manuel (Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803, USA)*

The evolution and spread of Plasmodium falciparum resistance to all front-line antimalarials is a serious threat to elimination, emphasizing the need to understand the underlying molecular mechanisms of the parasite's response to drug treatment. However, genome-wide analysis of transcription in P. falciparum has been shown to be highly dynamic, tightly regulated, and largely unperturbed by parasite exposure to antimalarial drugs. To date, most studies which examine gene expression changes after long exposure to common antimalarials have captured small or non-specific changes in mRNA abundance. With the application of rapid biosynthetic labeling of mRNA in P. falciparum (Painter et al. 2018), we can now directly measure the production of nascent RNAs, rather than mRNA abundance. As a result, rapid changes in transcriptional activity can be captured during time-course experiments with resolutions on the order of minutes. Here, we characterized the mRNA dynamics that occur in response to three common antimalarial compounds: atovaquone, chloroquine, and dihydroartemsinin. We have analyzed nascent mRNAs transcribed between 5 to 240 minutes post treatment and are able to detect both an immediate and an adaptive transcriptional response for each antimalarial tested. Immediate transcriptional responses are comprised of classic redox and stress response genes, whereas the adaptive response is specific to each compound and includes known drug targets. Our results also provide insight into the co-regulation of genes in response to antimalarial treatment allowing for the identification of common 5' regulatory motifs associated with the drug response gene network. This approach exploits rapid capture of nascent transcription following drug treatment, while providing a new and powerful tool to aid in the prediction of the mode-of-action of new antimalarials.

146: Cracking the coccidian egg: A molecular exploration of oocyst viability

Kruth, Perryn *(University of Guelph); R Barta, John (University of Guelph)*

The genus Eimeria includes dozens of parasites of significance to industrial farming. Coccidiosis, the disease caused by Eimeria spp., is an ongoing threat to commercial poultry. Costs are associated with control measures as well as loss of production efficiencies during subclinical or clinical infections. With resistance to once-effective drugs widespread, live vaccination with precise numbers of infective parasites of several Eimeria species has become important in controlling coccidiosis; effective vaccination requires accurate knowledge of oocyst viability. Determining viability currently requires costly and time-consuming live-bird infection trials that provide only limited, semi-quantitative data. Successful implementation of live vaccination programs would benefit from a more precise and timely measure of viability of constitutive parasites. We propose to use a PCR-based assay to determine viability of oocysts via assessment of their transcriptional activity. This poorly explored, dormant environmental stage of the parasite needs better characterization before an appropriate assay target can be identified. Preliminary experimentation has uncovered several potential transcriptional targets and has begun to shine light on the strategies used by the parasite to extend its window of infectivity that increases its opportunity for survival. Early data have shown up to a 20-fold decrease in specific assay target abundance in freshly killed oocysts compared to high-viability oocysts. With continued investigation of transcriptional activity of Eimeria spp. oocysts, we hope to uncover targets that will allow for increased assay specificity and range of application. More broadly, we hope to develop better understanding of the strategies used to persist in the external environment, allowing continuation of the lifecycle, that may be shared by other related parasites.

147: eIF2a phosphorylation in Trypanosoma cruzi is required for generation of trypomastigotes from intracellular amastigotes

Machado, Fabrício *(Federal University of São Paulo); Malvezzi, Amaranta M. (Federal University of São Paulo); Costa, Mirella (Faculdades Metropolitanas Unidas); Schenkman, Sergio (Federal University of São Paulo)*

In most eukaryotes, protein synthesis initiation is regulated by phosphorylation of the eukaryotic initiation factor 2 (eIF2) at serine 51, which stops overall translation by decreasing the availability of initiator tRNA to the ribosome. In trypanosomatids, the N-terminal eIF2α is extended, so a threonine at position 169 (T169) is the phosphorylated residue. Here, we evaluated the state of eIF2a phosphorylation during the life cycle of Trypanosoma cruzi, the protozoa parasite that cause Chagas disease. The total levels of eIF2α were found largely diminished in trypomastigote forms, which are infective and non-replicative. This is compatible with the decreased protein synthesis observed in these forms compared to epimastigotes and amastigotes, replicative stages found in the insect vector or in the cytosol of mammalian cells. More important, eIF2α is progressively phosphorylated in amastigote forms prior to differentiation into trypomastigotes. The role of this phosphorylation was assessed by overexpressing eIF2a with the phosphorylation site at T169 replaced by a non-phosphorylatable alanine residue. This mutation caused protein synthesis deregulation leading to a decreased formation of trypomastigotes that were also less infective and more susceptible to drug treatment. These results indicate that eIF2α expression and phosphorylation in intracellular amastigotes might be required for the translation arrest that occurs when infective T. cruzi trypomastigotes are formed.

148: Trypanosoma brucei antigenic variation in extravascular spaces

Mugnier, Monica *(Johns Hopkins Bloomberg School of Public Health); Bobb, Bryce; Rijo-Ferreira, Filipa; Figueiredo, Luisa*

Trypanosoma brucei lives an entirely extracellular life cycle in its mammalian host, facing a constant onslaught of host antibody. The parasite evades clearance by the host immune system through antigenic variation of its dense variant surface glycoprotein (VSG) coat, periodically “switching” expression of the VSG using a large genomic repertoire of VSG-encoding genes. Studies of antigenic variation in vivo have focused exclusively on parasites in the bloodstream, but recent work has shown that many, if not most, parasites are extravascular and reside in the interstitial space of tissues. This population has gone completely uncharacterized with respect to antigenic variation. We sought to explore the dynamics of antigenic variation in extravascular parasite populations using VSG-seq, a high-throughput sequencing approach for profiling VSGs expressed in populations of T. brucei. Our preliminary experiments suggest that trypanosomes in extravascular spaces are not cleared as efficiently as those in the bloodstream. This finding is in line with a model in which parasites “hide” from the immune system in tissue spaces, where a slower immune response provides them with more time to generate new antigenic variants. An analysis of VSG diversity in each space is consistent with such a model: more VSGs can be detected outside of the bloodstream while very few variants are unique to the blood. We are currently exploring the possible reasons why trypanosome clearance may be compromised in tissue spaces. Our results demonstrate that extravascular parasite populations are distinct from those in the blood and may play an important role in immune evasion during T. brucei infection.

149: Proteomic analysis reveals that the association of VSPs and kinases with lipid rafts in Giardia is important for encystation and host-parasite interactions

Grajeda, Brian *(University of Texas at El Paso); De Chatterjee, Atasi (University of Texas at El Paso); Pence, Breanna (University of Texas at El Paso); Polanco, Gloria (University of Texas at El Paso); Roychowdhury, Sukla (University of Texas at El Paso); Almeida, Igor (University of Texas at El Paso); Das, Siddhartha (University of Texas at El Paso)*

Lipid rafts (LRs) are nanoscale assemblies of cholesterol, sphingolipids (SLs), and proteins that form distinct liquid-ordered domains of the plasma membranes. LRs are involved in transducing extracellular signals and can undergo self-assembly and disassembly. Because raft-based signaling is critical for inducing growth and differentiation of many eukaryotes, and since Giardia switches form from trophozoites to cysts (called “encystation) during colonization in the small intestine, we hypothesized that signaling through raft microdomains is important for this encystation process. We used fluorescently conjugated cholera toxin B subunit (CTXB) to identify LRs in Giardia and conducted an examination by confocal and super resolution microscopy. The results indicate that raft-like structures in trophozoites are located in the plasma membranes and on the periphery of ventral discs. Pre-treatment of trophozoites with nystatin (a cholesterol-binding agent) and oseltamivir (a sialidase inhibitor) reduced CTXB reactivity, encystation, and attachment of trophozoites on mammalian cells. To understand the compartmentalization of protein in LRs, rafts and non-raft fractions were isolated from control- and drug-treated trophozoites by OptiPrepTM density-gradient centrifugation and subjected to the proteomic analysis using 2D-LC/MS/MS. We found that proteins of significant biological functions, including variant-surface proteins (VSPs) and kinases (Ser/Thr kinases, NEKs, etc.), are associated with raft fractions. The disassembly of LRs by nystatin and oseltamivir increased the partitioning of these proteins from the raft to non-raft fractions. These results indicate that raft formation and proper association of VSPs, kinases, and other proteins with LRs are important in maintaining various biological functions.
Currently, efforts are underway to develop compounds targeting LR assembly to treat giardiasis.

Poster Session C

Wednesday, September 12, 2018
3:00 pm – 5:00 pm

Poster Numbers 150 – 227

For Abstract see Session VIII, TT1
150: Mutations in the actin-binding protein PfCoronin confer resistance to Artemisinin in West African Plasmodium falciparum isolates
Sharma, Aabha

For Abstract see Session VIII, TT2
151: Mind the traffic: a role for trafficking in the cell stress response and artemisinin resistance in P. falciparum
Henrici, Ryan*; Zoltner, Martin; Hart, Melissa; Edwards, Rachel; van Schalkwyk, Don; Baker, David; Moon, Rob; Odom John, Audrey; Field, Mark; Sutherland, Colin*

For Abstract see Session VIII, TT3
152: Characterizing known translation inhibitors and drug candidates via Plasmodium falciparum whole cell extracts vs. S35 methionine incorporation: separating true 80S ribosome inhibition from artifact
Sheridan, Christine; *Garcia, Valentina; Ahyong, Vida; DeRisi, Joseph*

For Abstract see Session VIII, TT4
153: Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi
Berná, Luisa*; Rodriguez, Matias; Chiribao, Maria Laura; Parodi-Talice, Adriana; Pita, Sebastián; Rijo, Gastón; Alvarez-Valin, Fernando; Robello, Carlos*

For Abstract see Session VIII, TT5
154: Functional characterization of mitochondrial translation components in the early diverging eukaryote Toxoplasma gondii
Lacombe, Alice; *Tottey, J.; Ovciarikoval, J.; Courjol, F.; Gissot, M.; Sheiner, L.*

For Abstract see Session VIII, TT6

155: Antimalarial Drug Target PfATP4 is Present in Parasite Plasma Membrane as a Large Complex

Ramanathan, Aarti*; Vaidya, Akhil; Bhatnagar, Suyash; Morrisey, Joanne*

156: Transcription and localisation of sexual stage parasites in the P. chabaudi mouse malaria model.

Cunningham, Deirdre A. (The Francis Crick Institute)*; Deroost, Katrien (The Francis Crick Institute); Hosking, Caroline (The Francis Crick Institute); Manni, Sarah (The Francis Crick Institute); Vandomme, Audrey (The Francis Crick Institute); Lewis, Matthew (The Francis Crick Institute); Langhorne, Jean (The Francis Crick Institute)*

Asexual stage parasites sequester in the organs, thought to be a mechanism to avoid splenic clearance, and we have previously shown, in theP. chabaudimouse malaria model, that for the lungs and liver, such cytoadherence occurs concomitantly with the development of organ-specific pathology. Recent data for P falciparum showed that sexual stage parasites, gametocytes, sequester in the bone marrow, particularly in extravascular space. The P. chabaudi mouse malaria model, which gives rise to a chronic infection, particularly after vector transmission, can provide a unique system in which to address the effect on the performance of this tissue and to dissect the interaction partners of gametocytes in tissues, both with respect to vascular endothelium and extravascular space.We have developed transgenic parasites tagged with a variety of fluorescent proteins under the control of stage specific promoters, verified promoter specificity by RT PCR, and isolated asexual and sexual developmental stages by flow cytometry. Data will be presented on the transcriptome and tissue localisation of P chabaudi AS male and female gametocytes.

157: Structure of a novel dimeric lysine methyltransferase that regulates the motility of the human parasite Toxoplasma gondii

Pivovarova, Yulia (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)*; Liu, Jun (Department of Biology, Indiana University, Bloomington, IN 47405, USA); Lesigang, Johannes (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria); Hu, Ke (Department of Biology, Indiana University, Bloomington, IN 47405, USA); Dong, Gang (Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria)*

Lysine methyltransferases (KMTs) were initially associated with transcriptional control through their methylation of histones and other nuclear proteins, but have since been found to regulate many other cellular activities. The apical complex lysine (K) methyltransferase (AKMT) of the human parasite Toxoplasma gondii was recently shown to play a critical role in regulating cellular motility. Here we report a 2.1-Å resolution crystal structure of the conserved and functional C-terminal portion (aa289-709) of T. gondii AKMT. AKMT dimerizes via a unique intermolecular interface mediated by the C-terminal TPR (tetratricopeptide repeat)-like domain together with a specific zinc-binding motif that is absent from all other KMTs. Disruption of AKMT dimerization impaired both its enzyme activity and parasite egress from infected host cells in vivo. Structural comparisons reveal that AKMT is related to the KMTs in the SMYD family, with, however, a number of distinct structural features in addition to the unusual dimerization interface. These features are conserved among the apicomplexan parasites and their free-living relatives, but not found in any known KMTs in animals. AKMT therefore is the founding member of a new subclass of KMT that has important implications for the evolution of the apicomplexans.

158: Analysis of the Toxoplasma F-box Protein 1, FBXO1, Reveals That During Endodyogeny the Daughter Cell Scaffold Forms Before Centrocone Duplication

Baptista, Carlos G. (Department of Microbiology and Immunology, University at Buffalo); *Lis, Agnieszka (Department of Microbiology and Immunology, University at Buffalo); Dittmar, Ashley (Department of Microbiology and Immunology, University at Buffalo); Deng, Bowen (Department of Biochemistry and Molecular Biology, University of Georgia); West, Christopher (Department of Biochemistry and Molecular Biology, University of Georgia); Blader, Ira (Department of Microbiology and Immunology, University at Buffalo)*

F-box proteins (FBPs) are components of the Skp1, Cullin, F-box E3 ubiquitin ligase (SCF-E3) complex which is responsible for directing proteins for proteasome mediated degradation. FBPs are also important for SCF-E3 independent functions. While F-box proteins are conserved throughout eukaryotes, their functions in parasitic protozoa have not been investigated. The genome of Toxoplasma gondii predicts the presence of 16 FBPs (compared to 69 in humans). Five FBPs have been demonstrated to be important for parasite fitness in a recent genome-wide CRISPR screen. Here, we focused on the F-box protein TgFBXO1 because it is abundantly expressed in tachyzoites and because it has a very low fitness score. We endogenously tagged TgFBXO1 and confirmed that interacts with Skp1. Immunofluorescence assays revealed that TgFBXO1 is a component of the Inner Membrane Complex (IMC), which is an organelle that underlies the plasma membrane.

Early during endodyogeny, TgFBXO1 relocalizes to the developing daughter cell scaffold, which is the site where the daughter cell IMC forms and extends from. TgFBXO1 relocalization required centrosome duplication as it was blocked by TgMAPK1 inhibition. On the other hand, daughter cell scaffold localization of TgFBXO1 was dependent on TgRab11b, which is a small GTPase previously demonstrated to be require for IMC biogenesis. Importantly, relocalization to the daughter cell scaffold occurred before duplication of the centrocone complex, which is necessary to form functional spindle compartments. Finally, repeated failures at generating TgFBXO1 knockout strains or strains in which TgFBXO1 expression is reduced are consistent with TgFBXO1 being required for parasite fitness. This study is the first to assess FBPs in protozoan parasites and reveal a novel function for them during cell replication.

159: A secondary metabolite produced by a mollusk symbiont has activity against multiple apicomplexan parasites.

Driskell, I (Washington State University); *O'Connor, RM (Washington State University, Pullman, WA); Beaushaw, J (Washington State University, Pullman, WA); Bowden, G (1Washington State University, Pullman, WA); Lin, Z (University of Utah, Salt Lake City, UT); Schmidt, E (University of Utah, Salt Lake City, UT); Schafer, D (University of Arizona, Tucson, AZ); Riggs, M (University of Arizona, Tucson, AZ); Gimenez, F (Washington State University, Pullman, WA); Allred, D (University of Florida)*

Cryptosporidiosis is a common waterborne diarrheal disease that particularly impacts children and immunocompromised individuals, is caused by apicomplexan protozoans of the genus Cryptosporidium. Current treatment for cryptosporidiosis is limited to Nitazoxanide, which is ineffective in immunocompromised individuals. We looked to symbiotic bacteria of shipworms as a potential source of anti-parasitic compounds as marine natural products have gained acceptance as a potential source of pharmaceutical leads and therapeutic agents. Shipworms are marine bivalves that survive by borrowing into and consuming wood, a lifestyle entirely supported by their symbiotic bacteria. Analysis of shipworm symbiotic bacteria genomes revealed a significant commitment to the production of secondary metabolites, compounds that are likely to serve to protect the host and maintain the symbiosis. One of these compounds exhibited potent activity against the apicomplexan parasites Toxoplasma gondii and Cryptosporidium parvum. This compound, Tartrolon E (TrtE), was isolated by bioassay guided fractionation and was determined to have an EC50 in the low nM range against intracellular stages of multiple apicomplexan parasites. We verified TrtE potency in a neonatal mouse model of C. parvum infection, where the compound was highly effective at inhibiting infection. We have tested TrtE against the intracellular stages of the hemoparasites Babesia bovis and Theileria equi and show that the compound inhibited proliferation of both with EC50s of 11.2 nM and 342 pM respectively. Washout experiments demonstrated that only two hours of treatment were required for TrtE to inhibit intracellular growth of T. gondii. We are currently investigating the mechanism of action of TrtE utilizing Toxoplasma gondii and the drug affinity responsive target stability technique.

160: Strand-specific RNA Sequencing in Zoonotic Protozoan Pathogen Cryptosporidium parvum Suggests Widespread and Developmentally Regulated Long Non-coding RNA Transcription

Li, Yiran (University of Georgia)*; Baptista, Rodrigo (University of Georgia); Sateriale, Adam (University of Pennsylvania); Striepen, Boris (University of Pennsylvania); Kissinger, Jessica C (University of Georgia)*

Cryptosporidium is an apicomplexan protist identified as the second most prevalent diarrheal pathogen of infants in the world. To date, the regulatory elements orchestrating critical parasite processes remain largely unknown. It is becoming increasingly clear that long non-coding RNAs (lncRNAs) represent a missing regulatory layer across a broad range of organisms. Recently it was shown (Wang, Y. et al. 2016, PMID: 28007919) that specific parasitic lncRNAs from C. parvum were delivered into the host nucleus resulting in epigenetic transcriptional suppression of genes with effects on pathology. In this study, strand-specific RNA-seq data for both sporozoite and post-infection stages were used to perform a systematic annotation of lncRNA in C. parvum. The result revealed that at least 17% of protein-encoding genes have a putative overlapping lncRNA, indicating a more complex transcriptome and regulatory system than previously expected. The analysis of 221 high-quality lncRNA candidates revealed that their transcriptional expression was developmentally regulated with a generally positive correlation with upstream gene expression. Primary sequence and predicted structure conservation across many species in the genus were seen with some lncRNA candidates. Variation among species was noteworthy; one includes alternatively spliced isoforms in one species but not the others. In conclusion, lncRNA is a vital transcriptome component in Cryptosporidium with some evolutionary conservation and potential functions. PacBio Iso-seq is needed in the future to fully characterize the repertoire to solve the boundaries of overlapping transcripts because of the compact nature of this genome. This work has contributed to the initial characterization of the C. parvum non-coding transcriptome and may facilitate further insights into the roles of lncRNAs in parasitic development and parasite-host interaction.

161: Molecular dissection of the Plasmodium protease plasmepsin V

Polino, Alexander (Washington University School of Medicine)*; Goldberg, Daniel (Washington University in St. Louis)*

Upon invasion of an erythrocyte, P. falciparum exports hundreds of effectors into the host cytosol, drastically remodeling the host cell. Most of these exported proteins first pass through the parasite ER, where they are recognized and cleaved by the aspartic protease plasmepsin V (PMV), a promising drug target. A recent crystal structure of the P. vivax PMV revealed protein features that are unusual for an ER-resident protease and have unclear function. To interrogate the function of these features, we generated various PMV mutants and tested the ability of each to rescue depletion of a TetR-aptamer regulatable PMV, as well as their ability to cleave a fluorogenic substrate peptide in vitro.
We found that PMV lacking a well-conserved helix-turn-helix domain or an unpaired cysteine projecting into the active site retained the ability to complement PMV knockdown, suggesting these protein features do not play a role in essential PMV activity. Additionally, a poorly conserved large flexible loop on the protein surface is not essential for PMV function, raising the question of whether removal of these poorly structured loops will improve the enzyme's stability in heterologous expression systems. Taken together, our data represent a preliminary functional analysis of the protein features of a promising drug target and could guide further work development of inhibitors and investigation of this enzyme's biological role.

162: Phenotypic changes in Toxoplasma gondii when the Spindly O-fucosyltransferase is knocked out

Samuelson, John (Boston University)*; Bandini, Giulia (Boston University); Ichikawa, Travis (University of Gorgia); van der Wel, Hanke (University of Georgia); Haserick, John (Boston University); Costello, Catherine (Boston University); West, Christopher (University of Georgia)*

Nucleocytosolic O-GlcNAc transferase (OGT) is sensitive to the nutrient status of the cell and modifies Ser/Thr residues of many host proteins involved in transcription, translation, and signaling. Toxoplasma gondii encodes an OGT paralog, SPINDLY, which is absent in the host but present in Cryptosporidium, Dictyostelium, and plants. Here we show that T. gondii SPINDLY (TgSPY) is the nucleocytosolic O-fucosyltransferase that assembles fucose monosaccharides on Ser/Thr residues of >70 proteins involved in mRNA processing, nuclear transport (nucleoporins or NUPs), and signaling. These O-fucosylated proteins form assemblies, identified with the fucose-specific Aleuria aurantia lectin (AAL), in close proximity to the nuclear pore complexes. Stable knockout of the T. gondiispy gene in tachyzoites using CRISPR/Cas9 (spyKO) causes loss of AAL labeling of nuclei and loss of AAL binding to Western blots of total cell lysates. Complementation of the spyKO parasites with 3xMYC-tagged TgSPY, which localizes to the cytosol and nucleus, restores AAL-labeling of nuclei.The spyKO leads to a deficit in parasite growth on fibroblast monolayers, as judged from plaque assays. At a molecular level, we observed a decrease in the abundance and/or stability of a YFP reporter with a Ser-rich domain (SRD-YFP) and, to a lesser degree, Nup68-YFP. Both SRD-YFP and Nup68 are O-fucosylated in parent cells. In about 10% of the spyKO vacuoles, both an endogenous GPN loop protein with a 3xMYC-tag (O-fucosylated in wild type) and a YFP reporter with a nuclear localization signal (NLS-YFP), which is not a target of TgSPY, ‘leak’ from the nucleocytosol into the parasitophorous vacuole. Presently we are testing the virulence of spyKO in mouse and cat infections.

163: A genetic approach for understanding how malaria parasites correlate DNA replication and cytokinesis during the blood-stage life cycle.

Absalon, Sabrina (Boston Children's Hospital); *Dvorin, Jeffrey D (Boston Children's Hospital)*

During the intra-erythrocytic life cycle, Plasmodium falciparum divides by schizogony wherein the chromosomes do not condense and the nuclear envelope remains intact. Following multiple asynchronous rounds of nuclear replication (DNA segregation and nuclear envelope division), the multi-nucleated cell completes a single round of cytokinesis (budding) to form progeny called merozoites. Whereas, our previous studies have highlighted the essential role of the Merozoite Organizing Protein (PfMOP) for merozoite budding, the relationship between nuclear division and cytokinesis is less well understood. To investigate how DNA replication and cytokinesis are coordinated during the blood-stage life cycle of Plasmodium we took a genetic approach, focusing on PF3D7_1412100, the gene that encodes for the Mini Chromosome Maintenance Binding Protein (PfMCMBP). MCMBPs were first identified in human tissue culture cells in association with the MCM complex, a replicative helicase made of six subunits. Highly conserved among eukaryotes, the MCM complex is critical for the formation and elongation of the DNA replication fork. We identified the P. falciparum MCMBP ortholog and confirmed that it interacts with the MCM complex by co-immunoprecipitation.

Following inducible knockdown, we obtained a functional knockout of PfMCMBP and showed its essentiality for asexual parasite growth. While DNA replication appears normal, PfMCMBP-depleted parasites display abnormal nuclear morphology with scattered DNA and incomplete DNA segregation, highlighted by the presence of DNA bridges and nuclei of varied size. Following PfMCMBP-depletion, schizonts continue to mature and form daughter cells with abnormal cell body size. Despite the unusual nuclear architecture, schizonts with PfMCMBP-knockdown are not arrested in their development and continue their maturation resulting in the formation and egress of aberrant daughter cells. Using video microscopy, we observe that the newly egressed merozoites are noninvasive and die shortly after egress. Our results show that parasite segmentation and cytokinesis proceed despite abnormal nuclear division and size, suggesting that P. falciparum lacks a checkpoint for nuclear morphology prior to cytokinesis.

164: Toxoplasma Rop16III facilitates cyst development through host cell manipulations.

Kochanowsky, Joshua (University of Arizona); *Koshy, Anita (University of Arizona)*

Toxoplasma gondii is an intracellular protozoan parasite that establishes a life-long persistent CNS infection. Persistence in the CNS depends on Toxoplasma converting from a fast replicating form, the tachyzoite, to a slow growing encysted form, the bradyzoite. Most work on cyst development has focused on parasite genes that affect this transition, with less work done to define the host factors that influence the tachyzoite-bradyzoite switch. Two recent reports found that ROP16, a secreted polymorphic effector protein that affects host STAT signaling in a strain-specific manner, influenced cyst burden in vivo. As these studies identified the cyst effect in vivo, ROP16's effect on immune responses and parasite dissemination potentially confound these findings. To address this concern, we generated a ROP16 knock out in a type III strain and tested the ability of IIIΔrop16 to form cysts in an in vitro encystment assay. The IIIΔrop16 strain showed a decrease in cyst conversion compared to the parental strain. This defect can be complemented in trans, by co-infection with parental parasites, suggesting that ROP16III facilitates cyst development through manipulation of the host cell. We also engineered a IIIΔrop16 mutant that secretes Cre recombinase (IIIΔrop16:TCre) and infected Cre reporter mice that express a green fluorescent protein only after Cre-mediated recombination to track parasite dissemination to the CNS. Work with IIIΔrop16:TCre shows a decrease in dissemination to the CNS and a decrease in cyst burden. Current work is directed toward identifying what ROP16III domains are necessary and sufficient to enable cyst development and what host cell factors drive this ROP16III effect.

165: A Proteomic Approach Reveals the Molecular Manipulation of the Stress-Response Pathway in Trypanosoma brucei Induced by Down Regulation of the Mitochondrial Protein Translocase, Tim50

Chaudhuri, Minu (Meharry Medical College); *Singha, Ujjal (Meharry Medical College); Tripathi, Anuj (Meharry Medical College); Chaudhuri, Minu (Meharry Medical College); Rose, Kristie (Vanderbilt University); Sakhare, Shruti (Meharry Medical College); Pratap, Siddharth (Meharry Medical College)*

Trypanosoma brucei, the infectious agent for a deadly disease known as African trypanosomiasis undergoes various stresses during its digenetic life cycle. We previously showed that the parasite with a reduced level of TbTim50, a component of the translocase of the mitochondrial inner membrane (TIM), is more tolerant to oxidative stress. Here, we took a quantitative proteomic approach to understand the role of TbTim50 in cell homeostasis. Isobaric tagging followed by tandem mass spectrometry analysis revealed major rearrangements in proteins involved in metabolic processes and ribosomal biogenesis, due to changes in TbTim50 levels. Down regulation of TbTim50 specifically reduced the levels of cytochrome oxidase (COX) subunits, and increased levels of glucose transporters and trypanosome alternative oxidase. In support of this proteomic data we found that COX activity as well as ATP production by oxidative phosphorylation was reduced due to knockdown of TbTim50. Cell growth inhibition due to TbTim50 depletion was more pronounced in low-glucose than in normal medium. TbTim50 knockdown decreased mitochondrial membrane potential and increased the superoxide dismutase activity. Similar results were not observed upon TbTim17 knockdown, indicating that TbTim50 plays additional role besides protein translocation into mitochondria. Interestingly, TbTim50 knockdown increased the levels of glycosomal PIP39, an aspartate based C-terminal domain (CTD) protein phosphatase, similar to TbTim50. Double-knockdown of TbTim50 and TbPIP39 reduced tolerance of the parasite to oxidative stress and showed a synergistic effect on inhibition of cell growth in glucose depleted medium. Together, these results suggest that TbTim50 and TbPIP39 are connected in a signaling pathway to maintain cellular homeostasis.

166: Identification of a Novel Protein Phosphatase 1 Complex Involved in RNA Polymerase II Transcription Termination in Kinetoplastids

***Kieft, Rudo (University of Georgia)**; Zhang, Yang; Moran, Jose; Sabatini, Robert*

O-linked glucosylation of thymine in DNA (base J) represents an epigenetic mark regulating Pol II transcription termination within polycistronic gene clusters in Leishmania and T. brucei. The loss of base J at specific sites within gene clusters led to altered transcription termination and de-repression of downstream genes. The mechanism of transcription termination in kinetoplastids, including the role of base J, is unknown. However, the transition from transcription elongation to termination in humans and yeast involves desphorylation of the carboxy-terminal domain (CTD) of Pol II by PP1 protein phosphatase as part of the multimeric PTW/PP1 complex composed of regulatory subunits PNUTS, Tox4, and Wdr82 and PP1. Tandem affinity purification from L. tarentolea and mass spectrometry (MS) identified a similar complex of four associated proteins, including PP1 and potential homologues of PNUTS and Wdr82. The fourth protein (now called JBP3) contains a domain with homology to the J-DNA binding domain of JBP1 and is able to specifically bind J-DNA in vitro and thus, presumably a functional homolog of the Tox4 DNA binding protein. Reciprocal IP-MS in L. tarentolea and Co-IP analysis in T. brucei confirms complex association. We now refer to this complex as the PJW/PP1 complex and RNAi analysis supports its involvement in termination in T. brucei. Ablation of PNUTS, Wdr82 and JBP3 by RNAi leads to read-through transcription at an endogenous termination site as well as a tandem reporter construct integrated into the T. brucei chromosome. These data suggest a model where base J recruits the PJW/PP1 complex at termination sites and PP1 de-phosphorylates Pol II CTD, pausing elongation allowing efficient dissociation of Pol II.

167: Investigating ApiAP2 proteins with similar DNA binding specificities in Plasmodium falciparum

Bonnell, Victoria A. (Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16802)*; Josling, Gabrielle (Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16802); Russell, Timothy (Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16802); Painter, Heather (Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16802); Llinás, Manuel (Department of Biochemistry and Molecular Biology, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16802)*

The life cycle of Plasmodium parasites requires an intricate regulatory network to control gene expression. Although Plasmodium spp. possess genes encoding the canonical core eukaryotic transcriptional machinery, they also express 27 members of a plant-derived Apicomplexan AP2 (ApiAP2) family of sequence-specific DNA binding proteins. Since their first report, the characterization of ApiAP2 proteins has shed light on gene regulation at all stages of Plasmodium development. In this work, we focus on the functional characterization of a subset of four ApiAP2 proteins (PF3D7_0420300, PF3D7_0802100, PF3D7_1305200, PF3D7_1456000) that bind a similar CACACA DNA motif in vitro. Interestingly, half of these CACACA-binding ApiAP2 proteins have been proposed to be essential in P. falciparum and rodent models. We seek to understand how this subset of ApiAP2 proteins recognizes specific sequences throughout the genome during the intra-erythrocytic developmental cycle. To achieve this, we are using a combination of methods including chromatin immunoprecipitation and sequencing (ChIP-seq) and immunoprecipitation coupled with mass spectrometry (IP/MS) approaches to define gene targets and their interacting protein cofactors. In parallel, we will probe the DNA binding specificity in vitro using a next generation genomic context-dependent protein binding microarray (gcPBM) that contains all CACACA regions in the P. falciparum genome.
Furthermore, we are utilizing reverse genetics approaches such as gene knock-outs and knock-downs to determine the essentiality of these proteins to parasite development, and for successful cases, transcriptional changes will be evaluated by RNA-seq. Ultimately, the goal of this work is to functionally characterize these paralogous Plasmodium putative transcription factors by defining their differential roles in vivo.

168: Quantification of amino acid metabolism and protein synthesis in different strains of the apicomplexan parasite, Toxoplasma gondii

Monahan, Colleen (California State University - Long Beach); *Salladay, Ivan (California State University - Long Beach); Pace, Douglas (California State University - Long Beach)*

While much is known about the specific action of genes relating to Toxoplasma gondii virulence, less is known regarding its physiological state. Amino acid (AA) metabolism was quantified in Type I (RH) and II (PRU) extracellular tachyzoites to compare strain-specific physiological state. Free amino acid pool (FAAP) composition was determined through RP-HPLC, rates of AA transport and mass-specific protein synthesis (PS) were determined using 14C-labeled AAs. FAAP composition of both strains was similar. Transport rates of the basic AA, lysine, were similarly high in RH and PRU. Absolute rates of PS (using 14C-lysine) showed a strong correlation with strain-specific virulence with rates being 7-times higher in RH compared to PRU (3.5 and 0.5 fg parasite-1 hr-1, respectively). Similar rates of PS were also obtained using the aliphatic AA, alanine, as a tracer. Based on cellular protein content, rates of protein turnover were low at 1.32 and 0.21 % day-1 for RH and PRU, respectively. The importance of protein synthesis was further investigated using the PS inhibitor anisomycin. While PS was effectively inhibited, acute exposure to anisomycin had no effect on invasion efficiency, in concordance with the calculated low turnover rates. In total, there is a correlation between strain-specific virulence and extracellular PS. However, low turnover rates suggest a critical energy saving mechanism in both strains; allowing more energy to be directed towards invasion and replication. These results show that T. gondii tachyzoites have significant levels of nutrient transport and biosynthesis during the extracellular stage, however their low rates of turnover may have implications for the use of protein synthesis inhibitors as a treatment strategy.

169: Identifying protein-protein interactions for the P. falciparum Merozoite Organizing Protein

McGee, James P. (Boston Children's Hospital); *Absalon, Sabrina (Boston Children's Hospital); Rudlaff, Rachel M. (Harvard Medical School); Dvorin, Jeffrey D. (Boston Children's Hospital and Harvard Medical School)*

The human malaria parasite Plasmodium falciparum undergoes asexual replication during the blood stage via schizogony, a process in which the daughter parasites are formed by a specialized cytokinesis known as segmentation. In our previous study, we demonstrated that the P. falciparum Merozoite Organizing Protein (PfMOP, PF3D7_0917000) is essential for segmentation and gametocyte formation in blood stage parasites. To identify the protein-protein interactions for PfMOP, we are utilizing two different biochemical approaches. In the first approach, we utilized the promiscuous biotin ligase, BirA*, fused to PfMOP to identify proteins in close proximity to it (BioID). The top hits from the proximity labeling approach were evaluated for colocalization by immunofluorescence. In our second approach, we generated a new transgenic parasite strain with the high-avidity spaghetti monster tag fused to PfMOP. This parasite strain has facilitated co-immunoprecipitation experiments using an anti-HA antibody followed by unbiased mass spectrometry. Preliminary co-immunoprecipitation experiments have identified potential interactions with components of the glideosome as well as other multi-protein complexes in the cytoplasm. Ongoing studies are evaluating the validity and significance of the putative PfMOP-interacting proteins identified in our two biochemical approaches.

170: Role of Toxoplasma OTU-family deubiquitinases in the selection of cell cycle architecture and developmental transitions.

Sinai, Anthony P. (University of Kentucky College of Medicine); *Dhara, Animesh (Dept of MIMG University of Kentucky); Lynn, Bert (Department of Chemistry, University of Kentucky)*

Toxoplasma gondii exhibits 3 replication strategies, endodyogeny, schizogony and endopolygeny in a life-cycle dependent manner. Endoyogeny, is used by asexual stages (tachyzoites/bradyzoites), while a hybrid of schizogony and endopolygeny occurs in merozoites before to initiation of the sexual cycle.

Endodyogeny results in two progeny per cycle, while schizogony and endopolygeny result in multiple progeny being formed. This is achieved by the capacity of Apicomplexa to uncouple the nuclear cycle (DNA synthesis, mitosis/karyokinesis) from the budding cycle (cytokinesis). The targeted disruption of the Ovarian TUmor family deubiquitinase TgOTUD3A (TgGT1_258780) results in dysregulated replication with a subset of tachyzoites employing schizogony-like and endopolygeny-like strategies to produce 3, 4 and 5 progeny per cycle (Dhara et al. 2017 mBio). The partial implementation of replication strategies associated with the sexual cycle correlates with the transcriptional up-regulation of several merozoite specific genes. Furthermore, a majority of the Type I (RH) TgOTUD3A-KO tachyzoites express bradyzoite markers. In addition, the TgOTUD3A-KO, exhibited a marked increase in the expression of the closely related TgOTUD1B (TgGT1_237894) and TgOTUD1C (TgGT1_323200) which may compensate for the loss of TgOTUD3A. Thus the complex phenotypes associated with the TgOTUD3A-KO may be additionally be impacted by the induction of TgOTUD1 clade enzymes. Proteomic changes in the TgOTUD3A-KO are under investigation to establish the functional changes and identify the relevant substrate(s). Finally, as Type I (RH) parasites are developmentally incompetent, we have recapitulated the mutation in a Type II ME49 background. We are characterizing the impact on the cell cycle and developmental (bradyzoite and sexual cycle) markers toward functionally linking ubiquitin mediated mechanisms to both the cell cycle and developmental decisions.

171: Analyzing the function of branched-chain alpha-keto acid dehydrogenase (BCKDH) in Plasmodium falciparum

Munro, Justin T. (Chemistry Department, Center for Malaria Research (CMaR), The Pennsylvania State University); *Allman, Erik (Department of Biochemistry and Molecular Biology, Center for Infectious Disease Dynamics, Center for Malaria Research, The Pennsylvania State Universi); Llinás, Manuel (Department of Biochemistry and Molecular Biology, Center for Infectious Disease Dynamics, Center for Malaria Research, The Pennsylvania State Universi)*

Characterizing the metabolism of Plasmodium falciparum is relevant for drug development, by defining key differences between the parasite and the human host, as well as to attempt to circumvent the issue of antimalarial resistance.

P. falciparum can use glucose as a carbon source to complete the citric acid cycle (TCA) despite the localization of pyruvate dehydrogenase (PDH) to the apicoplast organelle. However, the development of ookinetes and gametocytes critically depends on ATP and NADPH that are generated by the TCA. We and others have implicated the role of branched-chain alpha-keto acid dehydrogenase subunit E1a (BCKDH; PF3D7_1312600) as a substitute enzyme for the conversion of pyruvate to acetyl-CoA. BCKDH is a mitochondrial protein that has been shown to have similar catalytic activity to PDH in vitro and in Plasmodium berghei and Toxoplasma gondii model organisms. Interestingly, the knockout of BCKDH in P. berghei restricts the parasite to grow in reticulocytes and hampers the maturation of oocysts in the sporozoite stage of the parasites. In this study, we examine the role of P. falciparum BCKDH during blood stage development. To accomplish this goal, we are using two complementary approaches. First, are pursuing a genetic approach in which we are generating bckdh knockdown and knockout parasites to determine their phenotype. Second, a chemical approach was also performed by artificially preventing pyruvate from entering the mitochondria by using UK-5099, a mitochondrial pyruvate carrier inhibitor, and measuring the effects of TCA pyruvate deprivation compared to untreated parasites using HPLC/MS based small molecule metabolomics. In summary, the reliance on BCKDH for the full P. falciparum TCA may pave the way for targeted drug treatments that prevent the complete maturation of gametocytes.

172: Molecular detection & isolation of Benzimidazole resistant Haemonchus contortus and evaluation of Anthelmintic activity of Herbal formulation

Qamar, Muhammad Fiaz MFQ (University of Veterinary & Animal Sciences)*; Ali, Kazim (University of Veterinary & Animal Sciences); Muhammad Arfan, Zaman (University of Veterinary & Animal Sciences); Muhammad, Younus (University of Veterinary & Animal Sciences); Ihtasham, Khan (University of Veterinary & Animal Sciences); Ehtisham ul, haq (University of Veterinary & Animal Sciences); Rabia, Tamkeen (University of Veterinary & Animal Sciences); Muhammad Imran, Rashid (University of Veterinary & Animal Sciences)*

This study central on molecular identification of Haemonchus contortus and isolation of Benz-imidazoles (BZ) resistant strains. The second part of the study was evaluation of efficacy of some medicinal plants against that resistant worms. Different abattoirs' of two geo-graphic regions of Punjab (Pakistan) were frequently visited for collection of worms. Out of 1500 (n=1500) samples that were morphologically confirmed as H. contortus, 30 worms were subjected to molecular procedures for isolation of resistant strains. Resistant worms (n=8) were further subjected to DNA gene sequencing. Bio edit sequence alignment editor software were used to detect the possible mutation, deletion, replacement of nucleotides. Genetic diversity were noticed and genetic variation existing in β-tubulin isotype 1 of the H. contortus population of small ruminants of different regions considered in this study. H. contortus showed three different type of genetic sequences. 75%, 37.5%, 25% and 12.5% of the studied samples showed 100% query cover and identity with isolates and clones of China, UK, Australia and other countries, respectively. Interestingly the neighbor countries such as India and Iran haven't much similarities with the Pakistani isolates. Thus, it suggests that population density of same genetic makeup H. contortus is scattered worldwide rather than clustering at a single region. BZ resistant H. controtus strains were treated with aqueous extracts of Nigella sativa and Tachyspermum ammi in vitro. Dose-and-time dependent response of the herbal extract against the resistant strains were recorded.

173: Erythrocyte Calcium-ATPase Activity Measured with Two Methods: Towards Novel Inhibitors as Antimalarials

Sims, Jeremiah N. (NIAID, National Institutes of Health); *Desai, Sanjay (NIAID)*

Ca2+ is an essential mediator of cellular activities in bloodstream malaria parasites, but little is known about this ion's transport at the infected red blood cell membrane. Efflux via the endogenous Ca2+ pump restricts uptake via a parasite-induced pathway and has been implicated in malaria disease severity. Because rigorous characterization of these transport activities would be facilitated by specific and potent inhibitors, we have developed a miniaturized fluorescence assay for Ca2+ efflux from intact red cells. We surveyed several Ca2+ indicator dyes and selected two for quantitative measurements. Human red cells were loaded with Ca2+ to steady-state levels using the ionophore A23187 under conditions optimized with kinetic measurements using 45Ca2+ flux and indicator dyes. Co2+ block and BSA extraction of the ionophore were also examined and optimized. Efflux via the Ca2+ pump was then tracked continuously, enabling studies of temperature-dependence and VO43- inhibition. Concordant results in tracer flux and fluorescent dye measurements yielded reliable estimates of transport at the plasma membrane. Early results from a high-throughput screen for Ca2+ transport inhibitors will be presented. These studies provide insights into Ca2+ trafficking in red blood cells and may lead to the development of novel antimalarial drugs that interfere with the multiple roles of this essential ion in Plasmodium spp.

174: Comparative Transcriptomics in L. braziliensis: disclosing differential gene expression of coding and putative non-coding RNAs throughout developmental stages

Teles, Natália (Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil)*; Ruy, Patrícia (Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil); Magalhães, Rubens (Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil); Dias, Leandro (Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil); Castro, Felipe (Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil); Myler, Peter (Center for Infectious Disease Research, 307 Westlake Avenue, Seattle,Washington, United States of America); Cruz, Angela (Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil)*

Leishmania (Viannia) braziliensis causes cutaneous and muco-cutaneous leishmaniasis. In this study the transcriptomes of procyclic promastigotes, Ficoll-purified metacyclics and axenic amastigotes of L. braziliensis (MHOM/BR/75/M2903) were analyzed and compared. Total RNA was used to determine the content and modulation of protein coding (mRNA) and non-coding RNA (ncRNA) throughout the developmental stages. Gene ontology analysis of the up- and down-regulated transcripts allowed identification of significantly enriched biological processes and previously unannotated genes were predicted. A computational pipeline was designed to uncover putative ncRNAs using 5 different ncRNA predictors (ncRNAs PORTRAIT, RNAcon, ptRNApred, snoscan and tRNAscan-SE). These bioinformatic tools allowed the identification of 11,372 putative ncRNAs in L. braziliensis, predicted by at least one of the ncRNA predictors. These transcripts were classified according to their size, genome location, direction and conservation among Leishmania species.

Differential expression (DE) analysis revealed 3,266 DE ncRNAs between procyclics and metacyclics; 3,058 between metacyclics and amastigotes and 4,380 between amastigotes and procyclics. Among the DE coding and ncRNAs, 216 coding and 295 ncRNAs were DE in all the three contrasts analyzed. Interestingly, the modulation of most of the coding (176) and ncRNAs DE transcripts (180) presented a profile of incremental levels from promastigotes to amastigotes. Thirty-five putative ncRNAs were subjected to Northern blotting, and one or more bands were observed in 22 of them. Knockout and tagging of 16 putative DE ncRNAs are underway using the CRISPR/Cas9 system. These transfectants will allow evaluation of possible functional roles of these ncRNAs in L. braziliensis. This work represents an outline of L. braziliensis transcriptome contributing to improve the understanding of coding and noncoding RNA content in the parasite. Financial support: FAPESP, Capes and CNPq

175: Population Genetics of Plasmodium falciparum and Plasmodium vivax in Islands of Vanuatu over a Decade

Dowd, Simone (Australian Defence Force Malaria and Infectious Disease Institute)*; Gray, Karen-Ann (Australian Defence Force Malaria and Infectious Disease Institute); Kaneko, Akira (Karolinska Instituet); Auliff, Alyson (Australian Defence Force Malaria and Infectious Disease Institute); Taleo, George (Ministry of Health Vanuatu); Vastergaard, Lasse (WHO); Auburn, Sarah (Menzies School of Health); Cheng, Qin (Australian Defence Force Malaria and Infectious Disease Institute)*

Vanuatu, located in the South Pacific Ocean, is an island nation consisting of approximately 80 islands governed as six provinces, with a population of 279,000 people. As Vanuatu has made remarkable achievements in reducing malaria transmission over the past decade the country has set its goal to eliminating malaria by 2030. It is the last vestiges of the malaria parasite population that will provide the main challenge to this goal. We collated 99 Plasmodium falciparum and 247 Plasmodium vivax parasite samples collected from 5 provinces over a 10 year period to examine the Plasmodium parasite population dynamics in country over this time of intensified control. Population genetics and diversity within and between the different locales and time points were examined. This was achieved using microsatellite markers. Data was analysed utilising the VivaxGEN database. Findings including Allele and Haplotype frequency, Expected Heterozygosity, Multiplicity of Infection, Population Differentiation and Linkage Disequilibrium for each parasite species on different islands will be presented. The data reflects progress made against malaria by the National Malaria Control Program of Vanuatu and will inform the ongoing elimination policy.

176: Identifying host components of the membrane enveloping intracellular Toxoplasma gondii

Cygan, Alicja (Stanford University)*; Branon, Tess (Stanford University); Ting, Alice (Stanford University); Boothroyd, John (Stanford University)*

As an obligate intracellular organism, Toxoplasma relies on commandeering host cell resources and modifying the intracellular milieu in order to establish a replicative niche (the parasitophorous vacuole, PV). Many critical host-parasite interactions occur at the host interface of the PV, the PV membrane (PVM).These include the recruitment of host organelles and cytoskeleton to the PVM, scavenging of essential nutrients, neutralization of host defenses, and transport of parasite effectors across the PVM. These crucial host-parasite interactions are mediated by both parasite and host proteins in or on the PVM, and while significant progress has recently been made in identifying new parasite proteins localized to the inside of the PV, to date, little is known about host proteins present on the PV, at the literal interface with the host cell. A comprehensive inventory of specific host proteins that associate with the PVM during infection is necessary to better understand the complex host-parasite interactions occurring at the PVM at a molecular level, and to identify novel and important host-parasite interactions. We are identifying host PVM-localized proteins using two complementary proteomic approaches: a broad, unbiased approach that exploits recent advances in protein proximity labeling using the promiscuous biotin ligase mutant, miniTurbo, and a targeted approach that identifies host PVM proteins that associate with known Toxoplasma PVM-localized proteins, MAF1 and MYR1. Results by both methods will be presented, emphasizing previously undescribed interactions occurring at this interface.

177: Compiling a minicircle genome of Trypanosoma brucei

***Yu, Tian (Boston University)**; Zhang, Liye (ShanghaiTech University); Monti, Stefano (Boston University); Aphasizhev, Ruslan (Boston University)*

Trypanosoma brucei is a parasitic protozoan that causes human African trypanosomiasis and livestock diseases. It harbors a highly complex mitochondrial genome composed of few catenated maxicircles and thousands of minicircles. Expression of most protein genes requires extensive U-insertion/deletion editing of maxicircle transcripts. These sequence changes are programmed by guide RNAs (gRNAs) transcribed from minicircles.
However, deep sequencing of small mitochondrial RNAs indicated that only 14.9% could be mapped to known minicircle sequences. These findings suggested that the T. brucei minicircle genome may be more complex than previously appreciated. To establish an unbiased and quantitative approach to profile the minicircle genomes, we applied PacBio Single Molecule Real-time (PacbioSMRT) platform to sequence 1kb minicircles isolated from purified kinetoplast networks. By clustering full-length minicircle reads, we identified 240 unique sequence classes, including 189 novel variants. Using small RNAs deep sequencing data as proxy, we estimate that the minicircle genome of T. brucei Lister 427 is 74.6 % complete. By juxtaposing small RNA positions and RNA polymerase DNA binding profile, we discovered that multiple gRNAs are independently transcribed from both strands. Furthermore, we identified conserved regions which likely function as promoters. To enable public access to our data, we constructed an annotated online database for the minicircle genome featuring gRNA locations and other features.

178: Development of a new optogenetic tool to study essential genes in Cryptosporidium parvum

Vinayak, Sumiti (University of Illinois-Urbana Champaign)*; Rose, Savannah (University of Illinois-Urbana Champaign); Gartlin, Brina (University of Illinois-Urbana Champaign)*

Cryptosporidium, a protozoan parasite, is a leading cause of diarrheal disease and death among infants and toddlers. Unfortunately, there are no effective drugs or vaccines to treat or prevent cryptosporidiosis. Nitazoxanide, the only FDA approved drug has limited efficacy and provides no relief in young children and immunocompromised individuals such as HIV/AIDS patients. Progress on the discovery of novel effective therapeutics has been hampered by the limited understanding of parasite biology due to (i) absence of methods for continuous culture of the parasite in the laboratory,(ii) poor animal infection models, and most importantly (iii) the lack of methods to genetically manipulate Cryptosporidium. Although we now have a powerful system to genetically engineer C. parvum, we are still lacking molecular tools to study essential gene function in the parasite. We have developed a new optogenetic tool that uses blue light for conditional protein degradation in C. parvum. We engineered a genetic construct that has the nanoluciferase gene fused to the blue light inducible domain. Transient transfection of C. parvum sporozoites with this fusion construct, and blue light illumination resulted in loss of luciferase expression. To validate this tool, we used the CRISPR/Cas9 genome editing system to create a stable transgenic parasite line that expresses the essential Calcium-Dependent Protein Kinase 1 (CDPK1) protein fused to the blue-light inducible domain. The correct 5’ and 3’ integration events at the cdpk1 locus was confirmed by PCR analysis of the stable transgenic oocysts. Ongoing experiments are directed towards optimizing the exposure time and other parameters to achieve a tight control of CDPK1 protein degradation. Future experiments are directed towards dissecting the phenotype associated with CDPK1 protein ablation, and applying this tool for studying function of other essential genes in Cryptosporidium.

179: Characterization the functional role of the Plasmodium falciparum AP2-G2 in gametocyotogenesis

Singh, Suprita (Penn State University); *Santos, Joana; Josling, Gabrielle (Penn State University); Orchard, Lindsey (Penn State University); Painter, Heather (Penn State University); Llinas, Manuel (Penn State University)*

Recent work has identified an ApiAP2 protein, AP2-G2, which plays a critical role in gametocyte maturation in the rodent malaria parasites Plasmodium yoelii and Plasmodium berghei. pbap2-g2 (PBANKA_1034300) knockout does not prevent commitment to sexual stages but rather prevents the development of mature gametocytes and also caused a decrease in the expression of genes expressed in either male or female gametocytes. Our goal is to characterize the function of the P. falciparum orthologue (PF3D7_1408200) of PbAP2-G2. To do this, we have generated both GFP-tagged PfAP2-G2 and pfap2-g2 knockout (KO) lines. The pfap2-g2 KO parasites proliferate normally in the asexual blood cycle but during sexual differentiation they arrest at Stage III and do not form mature gametocytes. In order to identify the genes that are regulated by PfAP2-G2 we performed transcriptome analysis throughout the 48-hour asexual life cycle comparing the knockout line to the wild-type parent. These data suggest that 206 genes changed significantly ($p<0.01$), of which 136 genes are up-regulated and 70 are down-regulated including several known gametocyte genes. We find that PfAP2-G2 is first expressed approximately 16 hours post-invasion and can be detected throughout the trophozoite and schizont stages. In order to identify the targets of PfAP2-G2 at the genomic level we performed chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) at both the trophozoite and schizont stages on the PfAP2-G2-GFP line using anti-GFP antibodies.

180: The Protein-Protein Interaction Landscape of the Apicomplexan Parasite Toxoplasma gondii

Stevens, Grant C. (University of Toronto)*; Swapna, Lakshmipuram (University of Toronto); Brand, Verena (University of Toronto); Boyle, John (University of Pittsburgh); Grigg, Michael (National Institute of Health); Emili, Andrew (Boston University); Parkinson, John (University of Toronto)*

While there have been many advances in understanding the biology of the human-infective Apicomplexan parasite Toxoplasma gondii, a majority of its proteins remain uncharacterized. Here we seek to elucidate the function of these proteins and examine their organization into macromolecular protein complexes through the generation of a high confidence protein-protein interaction network. Using a co-elution strategy, whereby extensive biochemical fractionation coupled with quantitative mass spectrometry is integrated with functional genomic datasets in both supervised and unsupervised machine learning, we have generated a final network that predicts 3017 interactions between 690 proteins. Clustering algorithms predict 75 protein complexes that recapitulate both conserved mammalian complexes utilized in training data and well-characterized canonical T. gondii complexes. The quality of these putative complexes is supported by the high co-expression of proteins within clusters, the nonrandom distribution of essential proteins within clusters and the congruency with available literary evidence. As such, we have predicted novel complexes linked to pathogenesis, the Inner Membrane Complex and metabolism. These clusters predict novel roles for uncharacterized hypothetical proteins, such as those present in a cluster of dense granule proteins, containing GRA14 and a MAF1 paralog, and a cluster of Inner Membrane Complex proteins, containing ISP3 and IMC19. Validation of these complexes is currently being performed using traditional molecular biology methods. Together these results demonstrate the capacity of our network to accurately reflect the protein landscape of T. gondii and serve as a community resource to readily generate and pursue testable hypotheses.

181: Use of micro RNA (mi RNA) therapy against Toxocara vitulorum in buffalo calves

Ayaz, Muhammad Mazhar (Bahauddin Zakariya University)

T. vitulorum is most commonly found in tropical and subtropical countries including Pakistan. It reduces the efficacy of animals up to 30-50% and some times leading to poor growth and death in young stock. Calves are infected via suckling and egg-producing adult worms will be present in their intestines about 3 wk after infection. These adult worms produce large numbers of eggs which are passed in calf feces onto the ground where they embryonate and become infective to other animals grazing on pasture. Mature worms remain in the calf for approximately 6 mo, at which time they are expelled. Males appear to be unaffected by the presence of hypobiotic parasites and are thought to be dead-end hosts.The ever increasing resistance against anthelemntics pose the young stock for heavy infestations and it is ever increasing demand for next generation drugs to be produced. miRNA are thought to be the next generation therapies against various parasitic diseases including Toxocariasis in Buffaloes/cattle too.

182: Biogenesis of Giardia intestinalis mitosomes

Voleman, Lubos (Department of Parasitology, Charles University)*; Tumová, Pavla (First Faculty of Medicine, Charles University); Doležal, Pavel (Department of Parasitology, Charles University)*

Mitosomes are the smallest evolutionary forms of mitochondria that evolved in eukaryotes adapted to anaerobic environments. This adaptation manifests as the absence of the mitochondrial genome and vast majority of the mitochondrial proteome, including the components of the mitochondrial division machinery. Here, we studied the dynamics of mitosomes in the human parasite Giardia intestinalis during interphase and mitosis and during differentiation into the cyst stage. We found that mitosomal division is restricted to mitosis, when both central and peripheral organelles divide in a unique and synchronized manner. During the segregation of the divided mitosomes, the subset of the organelles between two G. intestinalis nuclei had a prominent role. Surprisingly, despite the absence of the ERMES components, the division involves the association of mitosomes with the endoplasmic reticulum, a relationship commonly seen during the division of mammalian and fungal mitochondria.

183: Inducing massive sexual conversion in Plasmodium falciparum with a conditional pfap2-g over-expression system

***Llorà-Batlle, Oriol (ISGlobal)**; Michel-Todó, Lucas (ISGlobal); Cortés, Alfred (ISGlobal, ICREA)*

The study of Plasmodium falciparum transmission stages remains challenging, mainly due to the difficulties to obtain large amounts of synchronous gametocytes. To overcome this limitation, we developed a conditional over-expression system for pfap2-g – the master regulator of sexual conversion. Our strategy is based on the integration of a 5'cam-LoxP-hdhfr-3'hrp2-LoxP cassette upstream of the pfap2-g coding sequence using CRISPR/Cas9. Then, applying the DiCre system, the deletion of the LoxP-flanked elements can be induced with rapamycin, leaving pfap2-g under the control of the constitutive cam promoter. We applied this strategy to two different strains: E5, a 3D7 sub-clone with high basal sexual conversion levels; and 1.2B, a sub-clone that doesn't produce gametocytes. Upon induction we regularly obtain ≥90% of sexual conversion in E5, which to our knowledge is the highest sexual conversion rate ever observed under in vitro conditions. In 1.2B conversion was around 70%, demonstrating that pfap2-g activation is sufficient to trigger sexual conversion in a gametocyte non-producer sub-clone. The rapid and massive conversion enabled the transcriptional analysis of the largely uncharacterized sexually-committed schizont stages, which revealed several upregulated and downregulated genes in comparison to asexual schizonts. Among them we identified genes upregulated in committed schizont stages that are downregulated upon reinvasion, suggesting that they may play important functions in preparing parasites for sexual conversion. Currently, we are optimizing the system in the NF54 strain to perform gametocyte viability and standard membrane feeding assays to check the suitability of our system for the study of mosquito stages and high-throughput drug screenings.

184: Challenging the importance of Pantothenate and Coenzyme A biosynthesis during acute and chronic stages of Toxoplasma infection

***Lunghi, Matteo (University of Geneva)**; Krishnan, Aarti (University of Geneva); Soldati-Favre, Dominique (University of Geneva)*

Coenzyme A is an essential cofactor for a great range of metabolic functions. Its synthesis is conserved in all living organisms, and requires pantothenate (PAN, vitamin B5) as a precursor. Plants, fungi and bacteria possess the enzymatic repertoire for de-novo synthesis of PAN, while higher eukaryotes acquire it from dietary sources. In this context, the cyst-forming Coccidian subgroup of the Apicomplexa phylum encode the enzymes for PAN synthesis whereas the Haemosporida that include Plasmodium species salvage it from the host. T. gondii is a versatile parasite exhibiting the flexibility to scavenge as well as de-novo synthesize several key metabolites. To determine if T. gondii can salvage intermediates metabolites along the pathway to CoA production, we have targeted some key enzymes for genetic disruption. Conditional deletion of dephospho-CoA kinase, the enzyme responsible for the last step of CoA production, severely impacts on the lytic cycle of the parasite, confirming the essential nature of CoA synthesis. In contrast, the complete deletion of the gene coding for the PAN synthase (pantoate beta-alanine ligase) has no fitness cost for the in vitro growth of the parasite suggesting the existence of a transporter for PAN uptake. Remarkably, these parasite mutants exhibit a dramatic reduction of cyst burden in mice. This highlights the importance of the environmental niche and/or the developmental stage of the parasite for the access to host metabolites. While CoA de-novo synthesis is essential in T. gondii, both synthesis and scavenging routes for PAN are available.

185: New insights in the pir genes expression in Plasmodium chabaudi : a first step to decipher their function.

Vandomme, Audrey (The Francis Crick Institute)*; Talavera-López, Carlos (The Francis Crick Institute); Brugat, Thibaut (The Francis Crick Institute); Amis, Sarah (The Francis Crick Institute); Hosking, Caroline (The Francis Crick Institute); Cunningham, Deirdre (The Francis Crick Institute); Langhorne, Jean (The Francis Crick Institute)*

Subtelomeric multigene families of Plasmodium spp, such as the well-studied var gene family of Plasmodium falciparum, are thought to be involved in the pathogenesis of severe malaria, and in evasion of the host immune response. The other Plasmodium multigene families have been less well studied.
Plasmodium interspersed repeat (pir) genes are present in all Plasmodium species, and are related to the rifin and stevor genes in P. falciparum. Their roles in antigen variation, virulence or other functions have not yet been elucidated. However, in the experimental malaria model of Plasmodium chabaudi in C57BL/6 mice, the expression of many of the members of this gene family seems to be linked to an avirulent blood-stage infection.Here we have investigated expression of Plasmodium chabaudi pir (cir) genes during the 24hr intra-erythrocytic life cycle. We show clearly stage-specific cir gene expression at the RNA level, suggesting that cirs may have different functions at different stages of the cycle.The putative function of these stage-specific cir genes was then predicted using Deep Go, a new method that allows prediction of function based on amino-acid sequence and protein-protein interactome.
This approach highlighted several putative cellular processes in which CIRS could be involved, including gene regulation, phosphorylation and metabolism. We are currently using in situ tagging of cirs to localize their protein expression in the infected erythrocyte and to determine whether the localization of CIRS is compatible with their putative function(s).

186: Intricate hierarchical transcriptional control regulates Plasmodium falciparum sexual differentiation and maturation

Van Biljon, Riette (University of Pretoria); *van Wyk, Roelof (University of Pretoria); Orchard, Lindsey (Pennsylvania State University); Painter, Heather (Pennsylvania State University); Reader, Janette (University of Pretoria); Niemand, Jandeli (University of Pretoria); Llinás, Manuel (Pennsylvania State University); Birkholtz, Lyn-Marie (University of Pretoria)*

The extended gametocytogenesis of P. falciparum parasites remains an intriguing biological divergence within the genus, with commitment to sexual differentiation previously shown to be the result of an interplay between transcriptional regulation by epigenetic and ApiAP2 transcription factors and influenced by host derived lysophosphatidylcholine. Studies on understanding the parasite's commitment to gametocytogenesis have encompassed the overwhelming focus of research on sexual differentiation. Conversely, an in-depth analysis on the regulatory processes underlying progression through gametocytogenesis is still lacking. We contribute a detailed analysis of gametocyte differentiation that revealed distinct developmental transitions demarcating the start of gametocytogenesis and intermediate gametocyte development, resulting finally in maturation to produce the transmissible mature gametocytes. The study provides evidence for coordinated regulation of gene expression on a transcriptional level. We propose a model for regulation of gametocytogenesis in malaria parasites that involves active repression of gene sets mediated through epigenetics and RNA destabilization as well as active transcription of gene sets through successive ApiAP2 transcription factor activity. This data provides the most detailed framework of coordinated gene regulation events underlying development of P. falciparum gametocytes to date as a resource for the malaria community.

187: Impact of haemoglobin S on Plasmodium falciparum infected erythrocytes immune recognition in pregnancy-associated malaria

Chauvet, Margaux (UMR216 IRD-Universite Paris Descartes)*; Tetard, Marilou (UMR_S1134 INSERM); Cottrell, Gilles (UMR216 IRD - Universite Paris Descartes); Lohezic, Murielle (UMR216 IRD - Universite Paris Descartes); Pineau, Damien (UMR216 IRD - Universite Paris Descartes); Denoyel, Luc (UMR216 IRD - Universite Paris Descartes); Aussenac, Florentin (UMR216 IRD - Universite Paris Descartes); Roman, Jocelyne (UMR216 IRD - Universite Paris Descartes); Brossier, Emeline (UMR216 IRD - Universite Paris Descartes); Hanny, Marion (UMR216 IRD - Universite Paris Descartes); Luty, Adrian J.F. (UMR216 IRD - Universite Paris Descartes); Gamain, Benoît (UMR_S 1134 INSERM); Merckx, Anaïs (UMR216 IRD - Universite Paris Descartes); Migot-Nabias, Florence (UMR216 IRD - Universite Paris Descartes)*

Placental malaria is a critical health problem in tropical areas and associated with poor pregnancy outcomes. Pregnancy associated malaria (PAM) is caused by the adhesion and the sequestration of Plasmodium falciparum infected erythrocytes (IEs) in the placenta, mediated by the interaction of VAR2CSA with chondroitin sulfate A (CSA) on the syncytiotrophoblasts. During successive pregnancies, women develop a protective immunity against PAM by acquiring antibodies against VAR2CSA that inhibit the binding of IEs to CSA. Haemoglobin (Hb) S allele is highly prevalent in malaria endemic areas, resulting from the protection afforded by HbS heterozygous carriage (HbAS (sickle cell trait)) against severe P. falciparum malaria symptoms. HbAS IEs display lower amounts of VAR2CSA that are aberrantly presented, correlating with reduced cytoadhesive capacity to CSA. But little is known about the potential protection of HbS against PAM. We measured the ability of plasmas of HbAA and HbAS Beninese pregnant women to recognize VAR2CSA and parasite antigens on HbAA and HbAS IEs. On HbAA IEs, the parasite antigen recognition by plasmas did not change according to maternal HbS carriage. But considering both the haemoglobin genotype of the women and of the erythrocytes, we observed a lower parasite antigen recognition on HbAS IEs by HbAS plasmas than that on HbAA IEs by HbAA plasmas. Furthermore, 27% and 35% of women from our population group also carried glucose-6-phosphate dehydrogenase deficiency or a-thalassemia deletion, respectively. These other erythrocyte disorders coexist in populations affected by HbS, and also afford a protection against malaria. This study underlines therefore the necessity to investigate simultaneously the main erythrocyte disorders coexisting in a population group when studying immunological responses against PAM.

188: Comparative mapping of Plasmodium proteomes provides new insights into erythrocyte remodeling

Preiser, Peter (Nanyang Technological University); *Siau, Anthony (Nanyang Technological University); Hoo, Regina (Nanyang Technological University); Sheriff, Omar (Nanyang Technological University); Tay, Donald (Nanyang Technological University); Yam, Xue Yan (Nanyang Technological University); Loh, Han Ping (Nanyang Technological University); Mutwil, Marek (Nanyang Technological University); Wei, Meng (Nanyang Technological University); Sze, Siu Kwan (Nanyang Technological University)*

After invasion, Plasmodium extensively modifies the host-erythrocyte by secreting remodeling proteins in the parasitophorus-vacuole, the parasitophorus-vacuole-membrane (PVM), the host-cell cytoplasm and the erythrocyte membrane. However, without a common motif or structure available to identify these proteins, the Plasmodium remodelome remains elusive. Here, we mapped the proteome of five Plasmodium species. For this, infected red blood cell of P. yoelii, P. berghei, P. chabaudi, P. knowlesi and P. falciparum were fractionated and analyzed using quantitative TMT LC MS/MS revealing a proteome of ~2K to ~3K proteins per species. Differential analysis of the relative abundance of these proteins across the subcellular compartments allowed us to map their locations, independently of their predicted features. The proteome maps were further refined using gene orthology information and validated using genetic approaches. The analysis revealed that remodeling proteins encode for 8% to 17% of the parasite proteome and showed that while parasite proteins targeted to the periphery are mostly conserved across the genus, those exported beyond the PVM show significant lineage specificity. Reflecting the known differences between P. falciparum and rodent surrogate models, the distinct remodeling features noted for P. falciparum and rodent parasite support that these two lineages evolved differently from a common ancestor. Plasmodium knowlesi shares both exported protein families with rodent parasites as well as some clusters of PEXEL protein families with P. falciparum suggesting that other Plasmodium species have evolved a mixed approach. Our approach identified a significant number of remodeling proteins across the Plasmodium genus that likely represent key functions used by the parasite to promote survival and virulence. The presence of different remodeling features in the Plasmodium genus has important implications for the understanding of both common mechanisms and lineage-specific differences in the host-parasite interactions and could be crucial for the development of novel strategies against malaria.

189: Characterisation of an ApiAP2 factor associated with heterochromatin

***Carrington, Eilidh (Swiss TPH, University of Basel)**; Keller, Dominique (Swiss TPH, University of Basel); Toenhake, Christa G. (Radboud University); Bartfai, Richard (Radboud University); Voss, Till S. (Swiss TPH, University of Basel)*

Heterochromatin protein 1 (HP1) is a key factor in Plasmodium falciparum reversible gene silencing and marks heterochromatic areas found at the subtelomeric regions, internal chromosome islands and a small number of isolated genes. However, the mechanisms involved in establishing, maintaining and regulating heterochromatin are poorly understood in this parasite. Previously, we identified potential HP1 interaction partners by co-immunoprecipitation (co-IP) coupled with liquid chromatography tandem mass spectrometry and validated GDV1 as a key regulator of commitment to gametocytogenesis via its role in the destabilisation of heterochromatin (Filarsky et al., Science, vol. 359, 2018). A member of the ApiAP2 family of DNA-binding proteins, PF3D7_1456000 (PF14_0533), was also identified in this study as a potential HP1-interacting protein, suggesting a role in regulating the expression of HP1-associated genes or in organising heterochromatin. To investigate this putative interaction, we created parasite lines expressing endogenously tagged PF3D7_1456000 using CRISPR/Cas9 genome editing. PF3D7_1456000 co-localises with HP1 at the nuclear periphery as observed by fluorescence microscopy. Using chromatin immunoprecipitation-sequencing (ChIP-Seq) we found PF3D7_1456000 to be associated with heterochromatic regions throughout the genome, further supporting its interaction with HP1. To investigate the function of this ApiAP2 factor, we created a conditional knockdown cell line making use of the FKBP destabilisation domain (DD) system, which allows modulating DD-tagged protein expression levels by the addition or removal of Shield-1. Knockdown of PF3D7_1456000 expression causes no obvious morphology- or growth-related phenotypes. Current work is focused on comparing global transcriptional expression profiles of paired ON/OFF Shield-1 cultures over time to identify any differentially regulated genes. Additionally, we are generating new transgenic cell lines to further elucidate the role of this ApiAP2 factor and its interaction with HP1.

190: Screening Plasmodium falciparum sexual commitment

***Brancucci, Nicolas M. B. (SwissTPH, University of Basel)**; Hitz, Eva (SwissTPH, University of Basel); Voss, Till S. (SwissTPH, University of Basel)*

Malaria parasites depend on the formation of sexual precursor cells, gametocytes, for the successful transmission to the mosquito vector. The switch from asexual replication to the production of gametocytes is controlled by chromatin remodeling events and the subsequent de-repression of the ap2-g locus. Expression of the AP2-G transcription factor irreversibly primes cells for gametocyte differentiation in the next intra-erythrocytic cycle. We recently identified that Plasmodium falciparum controls the rate of AP2-G activation and gametocyte production in response to availability of the host factor lysophosphatidylcholine (lysoPC). The parasite uses lysoPC as a major source for choline to drive de-novo synthesis of the membrane lipid phosphatidylcholine. Sexual commitment and gametocyte formation is induced in response to limiting concentrations of lysoPC or choline. We used CRISPR/Cas9-dependent gene editing to generate parasite lines carrying fluorescent reporters at the C-terminus of AP2-G. Expression of the AP2-G-fusion proteins allows quantifying sexual commitment rates shortly after the cells have completed this cell fate decision. By combining this tool with knowledge gained from the link between parasite metabolism and ap2-g activation, we established a high content imaging-based assay that allows screening for conditions that either induce or inhibit sexual commitment. While the assay is amenable to systematic drug screening, it is also suitable to interrogate the role of specific candidate genes in sexual commitment by reverse genetics.

191: The dynamics of DNA replication during male gamete formation in Plasmodium falciparum

Matthews, Holly (Keele University); *Merrick, Catherine (University of Cambridge)*

The production of gametocytes involves some of the most unusual cell-biological phenomena in the lifecycle of Plasmodium. Male gametocytes, when triggered by the transition to the mosquito gut during a blood meal, are able to replicate their genomes 3-fold within ~10minutes and to undergo dramatic morphological changes, ultimately dividing to produce 8 flagellated gametes. This demands a speed of DNA replication and cell division unprecedented in eukaryotic gametogenesis. We have recently developed parasites in which nascent DNA replication can be labelled with bromodeoxyuridine (BrdU) (Merrick, Malar. J., 2015), allowing this process to be studied at high temporal and spatial resolution. Here, we have generated a gametocyte producing strain, NF54, that expresses thymidine kinase and incorporates BrdU, and have optimised this line for gametocyte formation and exflagellation. We have then measured the dynamics of DNA replication during male gamete formation at both the population and single cell levels. We observe that all male gametes that are viable to begin DNA replication do so within 5 minutes, but that the population as a whole continues replication through to 20 minutes. Formation of flagella is simultaneous with replication, and spindle poles appear in a significant minority of cells at 5 and 10 minutes, suggesting that genome division is concurrent with preparations for cytokinesis. Furthermore, in nascent gametes, a large amount of newly-replicated DNA appears to be dragged into the tails rather than appearing in the classical ‘head’ position. Perhaps most strikingly, many gametocytes elaborate flagella without any detectable DNA replication, as well as vice versa, suggesting a complete absence of the normal checkpoints that regulate successive phases of a classical cell cycle.

192: Signal peptide recognition particle-based protein targeting in mitochondria of Naegleria gruberi

Pyrih, Jan (Institute of Parasitology, Biology Centre CAS, Czech Republic)*; Cimrhanzlová, Kristýna (Institute of Parasitology, Biology Centre CAS, Czech Republic); Rašková, Vendula (Institute of Parasitology, Biology Centre CAS, Czech Republic); Kriegová, Eva (Institute of Parasitology, Biology Centre CAS, Czech Republic); Pánek, Tomáš (Life Science Research Centre, University of Ostrava, Czech Republic); Tsaousis, Anastasios (University of Kent, Canterbury,UK); Eliáš, Marek (Life Science Research Centre, University of Ostrava, Czech Republic); Lukeš, Julius (Institute of Parasitology, Biology Centre CAS, Czech Republic)*

In bacteria, the insertion of most inner membrane proteins is orchestrated via SecYEG-YidC complex, with their targeting being driven by an N-terminal signal peptide sequence. In mitochondria, this bacteria-derived machinery has undergone a dramatic restructuralization. The mitochondrial (mt) DNA of yeast codes for only a handful of genes, most of which specify highly hydrophobic proteins of the inner mitochondrial membrane. SecYEG is absent, and the membrane protein insertion is facilitated via Oxa1, a homolog of YidC. However, only limited information is available about other organisms. Here we focused on the free-living heterolobosean protist Naegleria gruberi, a close relative of the facultative parasite of human Naegleria fowleri. In order to decipher possible mechanism(s) by which sorting of those proteins occurs in the mitochondria, proteomic analysis of the whole organelle was performed.
Among 946 detected mitochondrial proteins, homologs of signal peptide recognition particle Ffh and signal recognition particle-docking protein FtsY were identified. Both proteins possess predicted mitochondrial targeting sequences and are targeted to the mitochondria when expressed in Trypanosoma brucei. Moreover, phylogenetic analysis suggested that the N. gruberi Ffh protein is of a-proteobacterial origin. Furthermore, we found a signal peptide resembling sequences on the N-terminus of the mt-encoded genes specifying hydrophobic proteins, whereas these motifs are absent in the soluble ones. These peptides were able to target reporter mNeonGreen fluorescent protein into endoplasmic reticulum of T. brucei as they were recognized by signal recognition particle in the cytosol. Combined, the data suggest that the bacterial signal peptide-based protein targeting system remains preserved in the mitochondria of N. gruberi and possibly other heteroloboseans and therefore represents a novel protein targeting system in eukaryotes.

193: Chemical Proteomic Identification and Characterization of the Druggable Thiolome in Toxoplasma gondii

Child, Matthew (Imperial College London); *Benns, Henry (Imperial College London); Bogyo, Matthew (Stanford University); Weerapana, Eranthie (Boston College); Tate, Edward (Imperial College London)*

Reactive cysteine residues display remarkable functional plasticity in proteins, mediating processes such as enzyme catalysis, and be the target of post-translational modifications such as palmitoylation. The reactivity of these cysteine thiols can be exploited by electrophilic inhibitors, making them excellent drug targets. During the lytic cycle of the protozoan parasite Toxoplasma gondii, reactive cysteines are associated with proteins involved in host-cell invasion, motility and cell morphology. However, a comprehensive understanding of the proteins dependent on such nucleophilic cysteine residues for their function is lacking in T. gondii. Using a quantitative mass spectrometry approach, we profiled the reactive thiolome in asexual stage T. gondii parasites and identified over 1,000 proteins. Enrichment analyses revealed 102 proteins containing highly-reactive cysteines, with diverse biological function, localization and PTMs. Notably, this included proteins of uncharacterised function, as well as proteins with established roles in parasite replication (Myosin F and Inner Membrane Complex Subcompartment Protein 2; ISP2), and organelle biogenesis (Apicoplast-associated Thioredoxin protein 1; ATrx1). To assess the importance of reactive cysteines on T. gondii biology, we have established a novel CRISPR-based point-mutation screen for systematic assessment of cysteine function, and also prioritized a selection of genes for classical validation using a conditional knockdown and complementation strategy. This study provides an initial insight into global thiol reactivity in the Apicomplexa, its functional contribution to parasite biology, and indicates that reactive cysteines have diverse roles in a number of cellular processes required for parasite survival.

194: New way to treat malaria based on cell biology

Wahlgren, Mats (Karolinska Institutet, Modus Ther. AB); *Leitgeb, Anna; Moll, Kirsten (Karolinska Institutet); Dondorp, Arjen (Moru)*

The drug sevuparin was developed from heparin because heparan sulfate and heparin are nearly identical, so the rationale was that sevuparin would act as a decoy receptor during malaria infection. A phase I study was performed in healthy male volunteers and sevuparin was found safe and well tolerated. A phase I/II clinical study was performed in which sevuparin was administered via short intravenous infusions to malaria patients with uncomplicated malaria who were also receiving atovaquone/proguanil treatment. This was a Phase I/II, randomized, open label, active control, parallel assignment study. Sevuparin was safe and well tolerated in the malaria patients. The mean relative numbers of ring-stage IEs decreased after a single sevuparin infusion and mature parasite IEs appeared transiently in the circulation. The effects observed on numbers of merozoites and throphozoites in the circulation, were detected already one hour after the first sevuparin injection. Here we report the development of a candidate drug named sevuparin that both blocks merozoite invasion and transiently de-sequesters IE in humans with P. falciparum malaria.

195: Investigating the essential nature of a citrate synthase-like protein in Plasmodium falciparum

Nicklas, Sezin K. (Drexel University College of Medicine); *Mather, Michael (Drexel University College of Medicine); Ke, Hangjun (Drexel University College of Medicine); Vaidya, Akhil (Drexel University College of Medicine)*

We previously demonstrated the dispensability of six of the eight TCA cycle enzymes for normal asexual blood stage metabolism and growth (Ke et al. 2015. PMID:25843709). Of these enzymes, the viability of a citrate synthase (CS) knockout transgenic parasite could have been due to a functional redundancy from a putative citrate synthase-like protein (CSL, Pf3D7_ 0609200). Despite belonging to the CS superfamily, CSL does not contain the conserved catalytic residues of CS. Multiple gene knockout attempts with CRISPR/Cas9 involving numerous guide RNAs yielded no viable parasites.

Ectopic tagged gene expression attempts were also unsuccessful. However, we were able to generate a transgenic parasite line in which the endogenous CSL locus was tagged with 3HA and aptamer repeats for TetR-DOZI regulated conditional expression as described by Ganesan et al. 2016 (PMID: 26925876). The protein is detectable at the correct size, and knockdown was verified by Western blot. Upon knockdown of CSL, these transgenic parasites ceased to grow. After 7 cycles, parasites that did arise had lost most of the aptamers, further supporting essentiality of the protein. Studies are underway to characterize this protein for its functional significance. Of interest to note, the citrate synthase (CIT1) of a ciliate organism, Tetrahymena thermophila polymerizes into filaments. As a structural protein in the cytoplasm, CIT1 is involved in formation of the T. thermophila's oral apparatus. Little is known about the Plasmodium oral apparatus, the cytostome. If CSL plays a similar role, this study

could shed light on cytostome formation in malaria parasites.

196: Antibodies to Plasmodium falciparum glutamic acid rich protein (PfGARP) protect against infection and severe disease

Raj, Dipak (Brown University); *Dasmohapatra, Alok (Brown University); Janiwali, Anup (Brown University); Kurtis, Jonathan (Brown University); Duffy, Patrick (National Institute of Health); Fried, Michal (National Institute of Health); Friedman, Jennifer (Hasbro Children's Hospital)*

Malaria caused by Plasmodium falciparum remains the leading single agent killer of children, yet the promise of an effective vaccine remains unfulfilled. Using our differential, whole-proteome screening method, we identified PfGARP as a parasite antigen recognized by antibodies in the plasma of children who are resistant but not by children who are susceptible, to malaria infection. PfGARP is an 80-kDa-parasite antigen expressed on the exofacial surface of early to late trophozoite-infected erythrocytes. Antibodies to PfGARP kill trophozoite-infected erythrocytes in culture and vaccination with PfGARP protects against P. falciparum challenge in non-human primates. In longitudinal studies, Tanzanian children with anti-PfGARP antibodies experienced 2.8-fold lower risk of severe malaria, and Kenyan adolescents and adults with anti-PfGARP antibodies had significantly lower parasite densities compared to individuals without these antibodies. By killing trophozoite-infected erythrocytes, PfGARP may synergize with other vaccines targeting hepatocyte invasion and erythrocyte invasion or egress.

197: Targeting a novel drug resistance pathway in P. falciparum

Fagbami, Lola (Harvard University) *A. Deik, Amanda K. Lukens. Clary B. Clish, Dyann F. Wirth*, Ralph Mazitschek*

Aminoacyl tRNA synthetases (aaRSs) are attractive targets for chemotherapeutic intervention in malaria because of the dependence of Plasmodium parasites on efficient protein translation in fast growing cells. Halofuginone (HFG), a derivative of the active ingredient of the Chinese medicinal plant Dichroa febrifuga, is a potent inhibitor of the cytoplasmic prolyl tRNA synthetase (cPRS) in P. falciparum. HFG treatment triggers a novel mode of drug tolerance in these parasites wherein intracellular proline levels are increased by 30 fold prior to any alteration in the target cPRS gene. This specific upregulation of proline levels in response to HFG treatment constitutes an unprecedented mode of drug tolerance and is a prerequisite for the development of genetic drug resistance.We sought to understand the molecular basis of this induced resistance phenotype by identifying the source of the increased proline using a multiplexed high-resolution mass-spectrometry (HRMS) based assay. By culturing HFG-induced parasites in the presence of orthogonally labeled proline and proline precursor amino acids (15N proline, 13C-15N arginine, and 13C5 glutamine), we observed predominantly 13C-15N-labeled proline, indicating that arginine is a major contributor. Using CRISPR/Cas9 technology, we have generated independent parasites lines that lack one of the enzymes in the arginine to proline metabolic pathway (arginase (PF3D7_906500) or ornithine d-aminotransferase (PF3D7_ 608800)). These knockout parasites are viable, indicating that these genes are not required for cell growth and are sensitive to HFG at a similar IC50 to the parental parasite. Selection with HFG is currently underway as well as confirmation of the metabolic deficiency. These experiments enable functional interrogation of this pathway in the ability of the parasite to establish HFG resistance.

198: Role of the RNA binding protein RBP42 in Trypanosoma brucei bloodstream form parasites

Das, Anish (Rutgers - New Jersey Medical School)*; Bellofatto, Vivian (Rutgers - New Jersey Medical School)*

In the absence of regulated transcription of individual genes, post-transcriptional processes that include mRNA maturation, localization, translation and decay, play pivotal roles in T. brucei gene regulation. Various RNA binding proteins (RBPs), by interacting with specific mRNAs, are the main effectors of this regulation. However, we are just beginning to understand how RNA binding proteins function in these parasites to accomplish the extensive and intricately networked gene expression patterns.T. brucei RBP42 is an essential RNA-binding protein. Previously we have shown that RBP42 mainly localize in the cytoplasm and is tightly associated with polysomes. Using HITS-CLIP analysis, we found that in procyclic T. brucei RBP42 preferentially associates within the coding sequence of mRNAs involved in cellular energy metabolism.Our current aim is to extend our knowledge of what role RBP42 plays in the metabolism of mammalian-infective bloodstream (BF) form T. brucei. To this end, we have generated a conditional knockdown cell line of BF T. brucei, in which drug resistance marker genes replace two endogenous alleles and a tagged RBP42 protein is expressed from an exogenous gene cassette in a tetracycline inducible fashion. We have used quantitative proteomics analysis, using iTRAQ methodology, to determine the changes in the proteome when RBP42 levels are decreased. Our preliminary analysis suggests RBP42 depletion leads to the specific alteration of many metabolic enzyme levels in the BF parasites. We are also performing iCLIP analysis to identify RBP42's in vivo mRNA targets in the BF stage of the parasite. A correlation of iCLIP data with proteomics data will shed light on RBP42's mode of action in parasite's gene expression. These data will be presented.

199: Assessment of the sensitivity and accuracy of the malaria Taqman array card in a field setting

Guler, Jennifer L. (University of Virginia)*; Kelly, Gillean; Dwomoh, Emmanuel; Warthan, Michelle; Pholwat, Suporn; Nsobya, Samuel; Mwanga, Juliet; Rosenthal, Phillip; Houpt, Eric; Kassaza, Kennedy*

The malaria Taqman array card (mTAC) is a compartmentalized card that partitions eight DNA samples into 384 different quantitative PCR reactions to assess 48 different P. falciparum genomic loci. The initial development of the mTAC showed excellent accuracy across 87 genotypes included (Pholwat et. al. 2017). Here, we assessed the limit of detection of the mTAC in a field setting and further evaluated its accuracy. Bloodspot DNA from density standards or patient samples from three collection sites in Uganda was run on the mTAC. Ct values were compared to estimate parasite levels in the patient samples. The minimum density detected was 21 parasites/ul; for this sample, 41% of mTAC assays were positive (of 48 total). For 100% mTAC assay detection, a parasite density of 176 parasites/ul was required. This limit of detection is on par with other PCR-based genotyping techniques even though the sample material was much lower (~0.4ul of DNA per assay). When we compared mTAC- and microscopy-based parasite densities, there was no correlation overall (R2 of 0.12), emphasizing the inaccuracy of microscopy for parasite quantification. When comparing mTAC genotyping results to Magpix-based results for a subset of loci, we detected decent concordance (3 of 4 loci compared yielded >78% agreement). Most of the disagreement was over mixed genotypes (both wild type and mutant present), which were prevalent in this region (~60% based on mTAC SNP barcodes). Lastly, we found that mTAC can accurately quantify copy number variations in the parasite genome. In addition to streamlined protocols and sample multiplexing, these characteristics will facilitate the use of mTAC in large-scale antimalarial resistance surveillance efforts.

200: Parasite specific labeling of N-acetylgalactosamine using a simple and robust ester-esterase pair system

Tomita, Tadakimi (Albert Einstein College of Medicine); *Wu, Peng (Scripps Research Institute); Weiss, Louis (Albert Einstein College of Medicine)*

Toxoplasma gondii is an obligate intracellular protozoan parasite of humans. Parasites persist in the CNS forming tissue cysts containing highly glycosylated cyst walls. Investigating cyst wall glycoproteins should shed light on mechanism(s) of cyst wall formation, parasite persistence, and host-parasite interactions. Isolating secreted glycoproteins from intracellular parasites is, however, quite problematic. For example, isolating and purifying parasites from host cells results in the loss of secreted parasite glycoproteins; however, if one includes host cells along with parasites when isolating glycoproteins the amount of abundant host cell glycoproteins overwhelm the analysis. To address these issues, we have established a specific labeling system where only parasite glycoproteins are labeled, but not those of host. Using a specific ester-esterase pair, e.g. porcine liver esterase (PLE), which can cleave a bulky carboxymethylcyclopropyl ester masking group. An azidosugar modified with this masking group can be unmasked by a parasite that is engineered to express PLE and incorporated into its glycoproteins, but cannot be unmasked by host cells lacking PLE. We have confirmed the utility of this approach using this PLE-ester sugar labeling system to examine O-GalNAc glycans in T. gondii. Parasites expressing PLE selectively labeled their glycoproteins, while the host cell glycoproteins remained unlabeled. By expressing PLE with stage specific promoters, we successfully labeled O-GalNAc glycoproteins selectively in either tachyzoites or bradyzoite. This simple and powerful ester-esterase pair system can be applied to T. gondii, an intracellular parasite, to deliver small molecules in a stage specific fashion to intracellular parasites allowing the study of various post-translational modifications as well as other biological processes without affect the host cell [Supported by NIH R21-AI127185 (LMW)].

201: Creation of a tissue culture model for Toxoplasma gondii sexual development

Di Genova, Bruno (University of Wisconsin-Madison); *Wilson, Sarah (University of Wisconsin-Madison); Spence, Jason (University of Michigan); Dubey, Jitender (United States Department of Agriculture); Knoll, Laura (Univeristy of Wisconsin-Madison)*

The sexual cycle of Toxoplasma gondii is limited to the feline small intestine where millions of oocysts are formed and subsequently excreted in their feces. The signaling required for differentiation of bradyzoites into merozoites is still unknown. Also, the definitive host specie specificity is unclear. The goal of this study is to recapitulate the T. gondii sexual cycle in tissue culture and comprehen its cellular and molecular mechanisms. Creation of ex vivo sexual development conditions will allow the contribution of specific genes to biological processes and redundant pathways to be rapidly analyzed. It will also allow for a molecular analysis of the complete lifecycle of a protozoan parasite, as T. gondii asexual development can already be performed in tissue culture.
Another benefit of tissue culture sexual development of T. gondii will be the stable production of a T. gondii vaccine vector against itself and other pathogens, such as Plasmodium. Recombinant T. gondii expressing circumsporozoite protein from Plasmodium yoelii or P. knowlesi provides protective immunity against malaria infection in mice and rhesus monkeys, respectively. A T. gondii vaccine vector produced as an oocyst will be ideal because it will be stable in virtually any environmental condition and it will be an oral inoculation.

202: Elucidating the mechanism of mitochondrial fission in Plasmodium falciparum

Mulaka, Maruthi (Drexel University College of Medicine); *Dass, Swati (Drexel University College of Medicine); Ke, Hangjun (Drexel University College of Medicine)*

Malaria is a huge global health burden and control of this disease has run into a severe bottleneck. Recent WHO statistics (World Malaria Report, 2017) suggests an increase of 5 million clinical cases and 16,000 deaths in 2016.

This rebound of malaria requires an urgent need to develop new interventions and antimalarial drugs. The mitochondrion of malaria parasite is a clinically validated antimalarial drug target. Inhibitors targeting parasite bc1 complex and DHODH are in clinical use or under development. Apart from pyrimidine biosynthesis, the mitochondrion participates in several essential processes such Fe-S cluster biogenesis and ATP generation (beyond the blood stages). During schizogony, from ring stage to mature schizont in erythrocytes, the mitochondrion divides from a small tubular structure to a complex branched network. In each schizogony, 8-32 merozoites are formed, the branched mitochondrion must divide and redistribute into single mitochondrion in each progeny. However, the mechanism of mitochondrial division and segregation is largely unknown. Recent reports showed that, proteins involved in mitochondrial fission/fusion (PfFis1, PfDRP1 and PfDRP2) are differentially expressed in erythrocytic stages of P. falciparum. We intend to investigate the role of PfFis1 (PF3D7_1325600) in parasite development and mitochondrial fission. In mammalian mitochondria, Fis1 involves in recruiting fission mediators to the mitochondrial membrane. Towards the functional investigation on role of PfFis1, we generated transgenic P. falciparum-Fis1 parasites with a TetR-DOZI-aptamer system. This system enables to control the expression of PfFis1 protein with anhydrotetracyclin (aTc). We are investigating the function of PfFis1 in blood and mosquito stage development. To monitor the mitochondrial fission in schizogony, we intend to generate P. falciparum parasites expressing fluorescent tags in mitochondrion and apicoplast.

203: Probing the organization and function of the Plasmodium falciparum parasitophorous vacuole membrane

Beck, Josh (Iowa State University); *Garten, Matthias (NIH); Heuser, John (NIH); Nessel, Timothy (Iowa State University); Roth, Robyn (Washington University School of Medicine); Zimmerberg, Joshua (NIH); Goldberg, Daniel (Washington University School of Medicine)*

Intraerythrocytic malaria parasites reside within a parasitophorous vacuolar membrane (PVM) that intimately overlays the parasite plasma membrane (PPM) and constitutes a key barrier between host and parasite. We recently found that EXP2, the membrane-spanning channel of the Plasmodium Translocon of EXported proteins (PTEX), serves dual roles as a protein-conducting channel in the context of PTEX and as a channel able to facilitate small molecule passage across the PVM. To better understand EXP2 distribution in the PVM, we generated a mNeonGreen fusion to the endogenous EXP2 protein, which often displayed a non-uniform, patchy distribution, frequently resolving into a distinctive “piebald” pattern in trophozoites with large EXP2-positive and -negative regions greater than a micron in diameter. Freeze-fracture replica immunolabeling corroborated this pattern, revealing a striking PVM organization into EXP2-decorated, protein-rich and protein-replete regions. To probe the content of these protein-rich PVM regions, we used the second generation BioID2 proximity-labeling system fused to EXP2. Proteomic analysis of biotinylated proteins identified many known PVM/PV proteins as well as some putative novel proteins. One of the most highly represented proteins was the integral PVM protein EXP1. To query EXP1 function, we adapted a CRISPR/Cpf1 genome editing system which we used to install the TetR-DOZI-apatmer system for conditional translational control at the exp1 locus. EXP1 knockdown resulted in a lethal defect accompanied by separation of the PVM and PPM. Our results suggest a role for EXP1 in PVM integrity and illustrate the power of BioID2 and Cpf1 (which utilizes an AT-rich PAM uniquely suited to the nucleotide content of the P. falciparum genome) for proximity protein identification and genome editing in P. falciparum.

204: Identification and targeting of male gamete specific factors involved in sexual reproduction during Cryptosporidium infection

English, Elizabeth (University of Pennsylvania)*; Sateriale, Adam (University of Pennsylvania); Beiting, Daniel (University of Pennsylvania); Striepen, Boris (University of Pennsylvania)*

Diarrheal diseases are the second leading cause of death in children under the age of five, accounting for approximately 11% of childhood deaths in 2010. Of the top four causes of pathogen-associated diarrhea in children 0-23 (rotavirus, Cryptosporidium, enterotoxigenic Escherichia coli, and Shigella), only Cryptosporidium lacks any vaccine or effective treatment. The only currently approved drug, nitazoxanide, has little to no efficacy above placebo in those most impacted by the disease, malnourished or immunocompromised children. Infection initiates when an individual ingests oocysts, typically from a contaminated water source. Following ingestion, infection is established in the small intestine, where parasites invade and replicate inside intestinal epithelial cells. Unlike many other parasites, Cryptosporidium is able to undergo both asexual and sexual reproduction within a single mammalian host. We hypothesize that the sexual portion of the lifecycle is necessary for both chronicity and spread of infection. For sexual reproduction to occur, male gametes must exit their host cell and then locate and fertilize intracellular female gametes. During this critical time, the male gamete is exposed to the host immune system and the environment of the gut, making it vulnerable to targeting by therapeutics. Here we aim to identify male-specific genes involved in finding and fertilizing female gametes. Using published studies on stage-specific gene expression in Plasmodium, along with our own in vitro expression data, we were able to compile a list of candidate male-specific genes. We then tagged and determined timing and localization of expression of each candidate gene. Candidate genes expressed specifically in sexual stages will be further evaluated for their role in sexual reproduction in Cryptosporidium.

205: C-Type Cytochrome Maturation and Function in Malaria Parasites

Espino, Tanya (University of Utah)*; Marvin, Rebecca (University of Utah); Nalder, Shai-anne (University of Utah); Sigala, Paul (University of Utah)*

Plasmodiummalaria parasites require heme as a metabolic cofactor, but our knowledge of heme utilization by parasites is limited to inhibitor studies of cytochrome b of the electron transport chain (ETC). To better understand the functional roles of heme-dependent mitochondrial cytochromes, we have comprehensively dissected the function and maturation of the ETC c-type cytochromes. These proteins are distinguished from other cytochromes by their covalent binding of heme, whose attachment requires mitochondrial holocytochrome c synthase (HCCS). In contrast to human cells, which express a single HCCS to mature cytochrome c and c1, parasites express two HCCS paralogs thought to be specific for cytochrome c or c1. We have reconstituted parasite cytochrome c maturation in E. coli and used this heterologous system to uncover that cytochrome c and c-2 are selectively matured by HCCS but not HCC1S, confirming the predicted specificity. UV-vis absorbance spectra of both cytochrome c paralogs suggest that these proteins have very different physical properties and that cytochrome c-2 has functionally diverged to perform a cellular role distinct from electron acceptance from Complex III. We used CRISPR/Cas9 to tag all five proteins with the aptamer/TetR-DOZI system for conditional expression. Knock-down (KD) of cytochrome c or c1 was lethal to blood-stage parasites but could be rescued by yeast dihydroorotate dehydrogenase (yDHOD), confirming the widely proposed model that parasites only require Complex III function to recycle ubiquinone for DHOD function. KD of cytochrome c-2 had no effect on parasite growth, consistent with a divergent function for this cytochrome that is not required for blood-stage parasites. KD of HCCS or HCC1S produced a substantial growth defect, consistent with non-overlapping roles for these synthases. These studies provide the first direct tests of heme utilization by blood-stage parasites and suggest new strategies to therapeutically target the mitochondrial ETC.

206: Imidazo[1,2b]pyridazines targeted against Plasmodium falciparum malaria block production of phosphatidylinositol-3-phosphate.

Safeukui, Innocent (University of Notre Dame); *Liu, Rui (University of Notre Dame); Pandharkar, Trupti (University of Notre Dame); Bhattacharjee, Souvik (University of Notre Dame); Liu, Haining (University of Notre Dame); Estiu, Guillermina (University of Notre Dame); Shirey, Carolyn (University of Notre Dame); Stahelin, Robert (University of Notre Dame); Mader, Mary (A Division of Eli Lilly and Company); Zink, Richard (A Division of Eli Lilly and Company); Margolis, Brandon (A Division of Eli Lilly and Company); Ochoada, Jason (A Division of Eli Lilly and Company); Montrose-Rafizadeh, Chahrzad (A Division of Eli Lilly and Company); Duffy, Sandra (Eskitis Institute for Drug Discovery); Avery, Vicky (Eskitis Institute for Drug Discovery); Burrows, Jeremy (Medicines for Malaria Venture); Bathrust, Ian (Medicines for Malaria Venture); Wiest, Olaf (University of Notre Dame); Grese, Timothy (A Division of Eli Lilly and Company); Miller, Marvin (University of Notre Dame); Haldar, Kasturi (University of Notre Dame)*

Phosphatidylinositol-3-phosphate (PI3P) functions in multiple organellar systems of P. falciparum malaria. It is synthesized by a single parasite phosphatidylinositol-3-kinase (PfPI3K). Further, PfPI3K binds PfKelch131, the major marker of resistance to artemisinins 2(that are frontline drugs for which we still have no replacement). Kelch13 resistance mutations increase levels of PfPI3K and its product PI3P 1. PI3P elevation induces artemisinin resistance and is predictive of resistance levels in clinical isolates, in engineered laboratory parasites and across non isogenic strains 1,3. Therefore, blocking PI3P production through inhibition of PfPI3K presents a strategy for anti-malarial drug discovery, but identifying a potent inhibitor series has remained elusive. Here we report inhibiting PfPI3K and PI3P production with imidazo[1,2-b]pyridazines. This series emerged from high throughput screening of compounds with diversity maximized with respect to the full compound collection at Eli Lilly and Company against the blood stages of P. falciparum.

Extensive structure activity and modelling studies combined in a

secondary screen yielded potent inhibitors (IC50 7-11 nM) with acceptable (30 fold) selectivity against human cell cytotoxicity. These inhibitors were in turn successfully validated using a parasite-stage specific reporter for PfPI3P, inhibition of parasite PI3P levels, activity against artemisinin sensitive and resistant strains as well as in an in vivo murine model. Together these data provide a foundation for design of inhibitors of PfPI3K to block PI3P production, an important anti-malarial target. 1.Mbengue, A. et al. A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. (2015). Nature 520, 683-687, https://doi.org/10.1038/nature14412. 2.Ariey, F. et al. A molecular marker of artemisinin resistant Plasmodium falciparum malaria. (2014). Nature 505, https://doi.org/10.1038/nature12876. 3. Bhattacharjee, S. et al. Remodeling of the malaria parasite and host human red cell by vesicle amplification that induces artemisinin resistance. (2018). Blood 131, 1234-1247, https://doi.org/10.1182/blood-2017-11-814665.

207: RTP4 is a negative regulator of IFN-I response during Plasmodium yoelii infection

He, Xiao (NIH/NIAID); *Wu, Jian (NIH/NIAID); Xia, Lu (NIH/NIAID); Peng, Yu-chih (NIH/NIAID); Tumas, Keyla (NIH/NIAID); Liu, Chengyu (NIH/NHLBI); Myers, Timothy G. (NIH/NIAID); Su, Xin-zhuan (NIH/NIAID)*

Infection of malaria parasites triggers dynamic and complex immune responses, and a coordinated response is required for successful resolution of a malaria infection. Recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) activates a series of signaling cascades, leading to production of cytokines such as type-I and type II interferons (IFN-I and IFN-II) and chemokines that mediate migration of immune cells to affected tissues. Previously, we performed a trans-species expression quantitative loci analysis (Ts-eQTL) using progeny from a genetic cross involving parasite strains that stimulate differential IFN-I responses and identified many putative IFN stimulated genes (ISGs). In this study, we performed functional screens to verify selected genes from the Ts-eQTL predictions. We then focused on an ISG gene called RTP4 (Receptor Transporter Protein 4), a probable chaperone protein that may facilitate trafficking and functional cell surface expression of some G-protein coupled receptors (GPCRs). Overexpression of RTP4 gene in 293T cell inhibited RNA induced RIG-I/MDA5 pathways and IFN-beta production, and knockdown or Knockout of RTP4 increase the expression of IFN by RNA stimulation. RTP4 binds to various molecules including STING, TRAF3, TBK1, and S6k1. RTP4 could also inhibit STING and TBK1 interaction and STING activation. To further characterize RTP4 in vivo, we generated RTP4 knockout mice. However, limited differences in parasite growth and mortality between wild type and RTP4-/- mice were observed after parasite infections. Further, we performed RNA-seq and found that in N67 parasite infection, PRR pathways in recognition of bacteria and viruses were activated in RTP4 KO mice, which suggests that RTP4 plays a role in host immune response to malaria infection. We are investigating molecular mechanism of RTP4 in regulating PRRs and IFN-I response during malaria infection.

208: MitoNEETS: mitochondrial redox sensitive iron sulfur cluster transfer proteins in malaria parasites

Dass, Swati (Drexel University College of Medicine)*; Mulaka, Maruthi (Drexel University); Morrisey, Joanne (Drexel University); Mather, Michael (Drexel University); Ke, Hangjun (Drexel University)*

MitoNEET is a newly discovered mitochondrial outer membrane protein that can transfer [2Fe-2S] cluster in a redox sensitive manner. Human mitoNEET is implicated to a spectrum of diseases including diabetes, cancer and neurological disorders. The crystal structure of various mitoNEET proteins reveals that this protein is a homodimer with each monomer harboring one [2Fe-2S] cluster binding geometry. Functionally, human mitoNEET has been linked to mitochondrial energy metabolism and iron homeostasis. We have found three mitoNEET like orthologues in Plasmodium falciparum (PfML1, PfML2 and PfML3). These proteins are localized to the mitochondrion in the asexual blood stages. Using CRISPR/Cas9 technique we have individually knocked out PfML1 and PfML2. However, PfML3 remains refractory to gene deletions with several repeated and modified attempts. In addition, the recent large-scale gene deletion studies carried out in P. falciparum and P. berghei were also unable to knock out ML3. We hypothesize that ML3 is the only essential mitoNEET like protein in asexual stages of malaria parasites. To further validate the essentiality of PfML3, we are attempting to generate a conditional knockdown line using the TetR-DOZI-aptamer system. In the knockdown parasites, we will analyze mitochondrial and cytosolic iron pools and other phenotypes. We also aim to express recombinant PfML3 protein to characterize its Fe-S cluster transfer activity in vitro. Through these experiments, we will identify the mechanism by which PfML3 regulates mitochondrial iron homeostasis and overall parasite health.

209: Characterizing novel Fis1 interactors to examine mitochondrial dynamics in Toxoplasma gondii

Jacobs, Kylie (Indiana University School of Medicine)*; Charvat, Robert (Indiana University School of Medicine); Garbuz, Tamila (Indiana University School of Medicine); Arrizabalaga, Gustavo (Indiana University School of Medicine)*

Toxoplasma gondii's mitochondrion is very dynamic and changes morphology throughout its life cycle, and in response to drug treatment or nutrient starvation. We have shown that challenge with the ionophore monensin induces gross alterations in mitochondrial morphology, which are completely reversible upon drug removal. Thus, we hypothesize that Toxoplasmaencodes for proteins that regulate mitochondrial dynamics. We focused our attention on TgFis1 (TgGT1_263323), a homologue of the mitochondrial fission protein Fis1 which recruits other proteins for mitochondrial division. We determined that TgFis1 localizes to the outer mitochondrial membrane (OMM) and ectopic expression of TgFis1 lacking the transmembrane domain acts as a dominant negative and results in polyploidy and disruption of mitochondrial morphology. As Toxoplasmaappears to lack some of the proteins expected to act in conjunction with Fis1 during mitochondrial fission, we identified putative interactors using both a yeast two-hybrid screen and immunoprecipitation assays. One protein with no known homology was identified through both approaches. This putative interactor does not have a transmembrane domain or any other feature that would predict interaction with membranes or localization to the mitochondrion. Nonetheless, we have shown that its association with the mitochondrion is dependent on TgFis1Based on interaction data and localization, we named this protein Fis1 Interacting Protein 1 (FIP1). Importantly, knockout of FIP1 results in a significant disruption of parasite propagation. Detailed phenotypic analysis of the FIP1 mutant strain is still ongoing and will be presented. Our discovery of a novel protein that interacts with the mitochondrial fission machinery will shed light on the regulation of mitochondrial dynamics.

210: Optimization of an approach to detect copy number variation in a single parasite genome

Liu, Shiwei (University of Virginia); *Guler, Jennifer (University of Virginia)*

Copy number variation (CNV) is suggested to be a major contributor for the development of antimalarial resistance in Plasmodium falciparum. However, the mechanisms behind CNV formation are not fully explored in the parasite. CNV profiling on a single cell level can help identify conditions that trigger their formation. Uniform whole genome amplification is important for single-cell CNV detection. Most amplification methods, such as PCR-based methods and multiple displacement amplification (MDA), are prone to bias and obscure real CNV signals. Another approach termed MALBAC, or multiple annealing and looping-based amplification cycles, exhibits improved coverage and uniformity due toa random primed “quasi linear” pre-amplification phase followed by exponential amplification with limited PCR cycles. Some challenges with this amplification approach include bias of GC-rich sequence and nonspecific amplification, especially when starting material is extremely low. For these reasons, amplifying the small (~25 femtograms), extremely AT-rich genome from single P. falciparumparasites using MALBAC requires optimization.Here, we trial a number of modifications to improve use with this organism. We incorporate PCR additives and redesign degenerate primers to improve amplification in AT-rich regions of the parasite genome. Additionally, we use a novel thermostable polymerase with strand displacement activity and limit the reaction volume to reduce contamination risk. The optimized protocol will be employed to analyze CNVs on a single parasite level, which will allow in depth investigations into mechanisms that lead to their formation in the parasite genome.

211: Pyruvate Kinase II is Required for Apicoplast Maintenance

Swift, Russell (Johns Hopkins School of Public Health)*; Keutcha, Cyrianne (Johns Hopkins School of Public Health); Liu, Hans (Johns Hopkins School of Public Health); Prigge, Sean (Johns Hopkins School of Public Health)*

While the apicoplast is essential for Plasmodium falciparum, blood-stage parasites can survive without the organelle as long as the isoprenoid precursor isopentenyl pyrophosphate (IPP) is supplemented. While this demonstrates that IPP is the only indispensable product of the organelle during this stage, other pathways within the apicoplast play accessory functions to its production, or are involved in the maintenance and replication of the organelle. To help investigate these essential pathways we developed a mevalonate-dependent metabolic bypass of the apicoplast expressing enzymes from an alternative isoprenoid precursor pathway. This parasite line allows us to delete essential nuclear-encoded apicoplast-specific genes and characterize the phenotypes associated with their loss. Through a series of genetic knock-outs we have determined which proteins involved in carbon import and metabolism within the apicoplast are essential for the maintenance and replication of the organelle. Central to this process is pyruvate kinase (PyrkII), which uses phosphoenolpyruvate and NDPs as substrates to generate pyruvate and NTPs, providing the only known source of pyruvate and ATP within the organelle.

Deletion of the upstream inner triose phosphate transporter (iTPT) that imports three-carbon phosphate sugars, including phosphoenolpyruvate, replicates the phenotype seen with the deletion of PyrKII. However, the deletion of genes within the pathways that are reliant on pyruvate (FasII and MEP) did not result in organelle disruption, suggesting that the essential activity of PyrkII is the generation of NTPs. We tested the substrate specificity of PyrkII and found that it was active against all NDPs, indicating that it may play an important role in the generation of NTPs within the apicoplast. We have additionally generated lines that will allow us to further investigate the role of PyrKII by knocking down (tetR-DOZI) or mislocalizing (conditional localization domain) the protein away from the organelle and tracking the relative loss of organellar genome transcripts.

212: Reticulocyte conditioned media stimulates gametocytogenesis in Plasmodium falciparum culture

Evans, Rachel M. (Johns Hopkins Bloomberg School of Public Health)*; Sullivan, David (Johns Hopkins Bloomberg School of Public Health); Wu, Mengyao (Huashan Hospital of Fudan University, and Johns Hopkins University School of Medicine); Cheng, Linzhao (Johns Hopkins University School of Medicine); Gao, Yongxing (Johns Hopkins University School of Medicine)*

Malaria infection by Plasmodium falciparum continues to afflict millions of people worldwide, with transmission maintained by the definitive host mosquito. Transmission is dependent upon mosquito ingestion of the gametocyte stage of the parasite. These sexually committed stages develop from the asexual stages, yet the factors behind this transition are poorly understood. Further, in vitro studies have revealed that extracellular factors present in different media and cellular environments influence gametocytogenesis. We hypothesize gametocytogenesis is induced through molecular factors found in parasite- or reticulocyte-rich environments. We have demonstrated that human induced pluripotent stem-cell derived erythrocyte conditioned media significantly influences gametocytogenesis in an NF54 strain P. falciparum infection. We adapted a gametocyte-specific quantitative Pfs16 promoter luciferase assay to examine the effects of media environment on gametocyte development. Both conditioned and non-conditioned terminal maturation media (TM media) significantly increased gametocyte numbers on days 4 and 6, counted via blood film and luciferase assay. We then tested the effects of known supplements found in TM media, as well as several other molecules that have been previously implicated in gametocyte development. We found that heparin, insulin, erythropoietin, holo-transferrin, heme, cyclic AMP, oleic acid, palmitic acid, bilirubin, and biliverdin have no significant effect on gametocytogenesis. We found that low media pH appears to increase gametocyte development after day 3 of culture, and that physiologically relevant levels of lactic and uric acids induce Pfs16-controlled luciferase at day 3. In addition, lactic acid and uric acid appear to influence gametocytogenesis in a dose-dependent manner. We will continue to test various acids produced by parasites or reticulocytes in a dose and time dependent to determine their effects on gametocytogenesis.

213: Stochastic Gene Expression in Plasmodium falciparum Asexual Stages: A Single Cell Transcriptomics Approach

Tripathi, Jaishree (Nanyang Technological University); *Zhu, Lei (Nanyang Technological University); Bozdech, Zbynek (Nanyang Technological University)*

P. falciparum is the most virulent malaria parasite which causes considerable morbidity and mortality worldwide annually (1). Studying transcriptional heterogeneity in seemingly clonal parasite population will lead to better understanding of biological processes, such as, stochastic gene expression, cell fate determination and response to external stimuli such as treatment with antimalarials. Here, we present an optimized non-exponential whole transcriptome amplification (WTA) method which allows detection of up to ~1700 transcripts per parasite (~30% of the transcriptome) and has higher reproducibility and sensitivity than the widely used SMART-seq2 WTA protocol. We combine this optimised non-exponential WTA method with high throughput Next Generation Sequencing (NGS) to measure cell-to-cell variability in gene expression in P. falciparum asexual stages. References:World Malaria Report. WHO (2016)

214: Functional characterization of a novel P. falciparum serine hydrolase important for ring stage formation and early intraerythrocytic development.

Ridewood, Sophie (The Francis Crick Institute)*; Annett, Dara (The Francis Crick Institute); Deu, Edgar (The Francis Crick Institute)*

Serine hydrolases are involved in a variety of biological and metabolic processes. However, this enzyme family is poorly annotated in Plasmodium spp., and very little is known about the role of these enzymes in parasite development. Our lab has recently used chemical biology approaches to profile the activity of all metabolic serine hydrolases throughout the asexual life cycle of P. falciparum. Interestingly, we have identified several enzymes that seem to be specifically activated at merozoite stage, possibly indicating roles in egress, invasion, or early intraerythrocytic development. Here, we show that the activity of one of these enzymes, which we have named S9C, is important for early ring development.Using the DiCre conditional KO system, we have shown that S9C-deficient parasites are able to egress and invade RBC normally, but ring stage parasites exhibit a stunted morphology and a severe delay in intraerythrocytic development. Conditional mutation of S9C into an inactive enzyme induces the same phenotype indicating that S9C activity is important for early ring formation and/or development.S9C has previously been shown to be N-myristoylated, and our localisation studies suggest that in mature schizonts and merozoites S9C localises to the cytoplasmic side of the rhoptries. Interestingly, in newly invaded RBCs S9C has a peripheral membrane localisation. Overall, our studies indicate that S9C activity is important for the correct formation of ring-stage parasite, and we propose that S9C might be involved in the formation of the parasitophorous vacuole and/or in nutrient acquisition. Electron microscopy studies are currently underway to characterise the phenotype associated with the loss of S9C, and we are also expressing this enzyme to determine its biochemical function.

215: ER resident PfGRP170 is an essential protein in the human malaria parasite, Plasmodium falciparum

Kudyba, Heather M. (University of Georgia); *Cobb, David W. (University of Georgia); Florentin, Anat (University of Georgia); Fierro, Manuel (University of Georgia); Rodriguez, José (University of Georgia); Ravishankar, Rajani (University of Georgia); Muralidharan, Vasant (University of Georgia)*

The vast majority of malaria mortality is attributed to one parasite species: Plasmodium falciparum. Asexual replication of the parasite within the Red Blood Cell (RBC) is responsible for the pathology of the disease. There are currently no effective vaccines against P. falciparum and drug resistance has emerged for all clinically available drugs, thus making malaria research extremely important. In Plasmodium, the endoplasmic reticulum (ER) is a uniquely complex, poorly understood organelle. We are therefore interested in uncovering proteins, which regulate and maintain ER biology in the parasite. One group of proteins possibly governing these processes are ER chaperones. In other eukaryotes, ER chaperones assist with protein folding and unfolding, the crossing of biological membranes, ER stress, and protein trafficking. We know very little about the roles that ER chaperones play in Plasmodium, with most of the information we have based on sequence homology to other organisms. We have generated conditional mutants for PfGRP170, a previously uncharacterized P. falciparum ER chaperone, allowing us to interrogate its biological function. These conditional mutants were isolated using flow cytometry and proper integration was confirmed using a Southern blot. Western blot analysis demonstrates that this fusion protein is expressed in the parasites and immunofluorescence assay indicate that the protein localizes to the parasite ER in all stages of asexual development. Unlike its homolog in other eukaryotes, our data show that PfGRP170 is essential for parasite survival with RBCs. Life cycle analysis revealed that knockdown results in parasite death in early schizogony. The protein is

required for surviving a brief heat shock, suggesting that PfGRP170 is essential during febrile episodes in the host. Proteomic analysis revealed that PfGRP170 interacts with many proteins exported to the host RBC, however no clear defects in trafficking of exported proteins have been observed. Our data show that PfGRP170 interacts with the Plasmodium homolog of BiP, an essential chaperone in mammalian cells. PfGRP170 knockdown leads to the phosphorylation of EIF2-alpha, which is an indicator of ER stress. Our data show that the chaperone PfGRP170 is an essential protein required for key ER functions in the clinically relevant asexual stages.

216: An ER-resident Hsp40 is required for the asexual development of the malaria parasite P. falciparum.

Cobb, David W. (University of Georgia); *Kudyba, Heather M. (University of Georgia); Bruton, Baylee (University of Georgia); Muralidharan, Vasant (University of Georgia)*

Malaria remains a significant global health burden. The parasitic disease kills hundreds of millions of people every year, with infection by Plasmodium falciparum associated with the most severe cases of malaria. All of the clinical symptoms of malaria result from the asexual replication of Plasmodium parasites within human red blood cells (RBCs); thus, an understanding of the mechanisms used by the parasite to survive within the RBC is critical. The Endoplasmic Reticulum (ER) is an organelle central to parasite biology, and its function is required for parasite survival. The ER serves as the starting point for protein trafficking to other organelles and to the host RBC, and stress response signaling from the ER is associated with parasites surviving treatment with the frontline anti-malarial Artemisinin. ER-resident chaperones support ER function and are therefore ideal candidates for exploring the parasite's biology. To this end, we have generated a conditional knockdown parasite line for PfJ2, a putative ER-resident Hsp40 expressed throughout the asexual cycle. Using this parasite line, we have confirmed that PfJ2 is an ER-resident protein and is essential for parasite survival inside the RBC. Specifically, knockdown of PfJ2 results in delayed parasite development during the trophozoite stage before failure to complete schizogony to form new invasive parasites. ER functions, such as protein trafficking and stress response, will be assayed during PfJ2 knockdown to investigate the chaperone's role in these processes. Additionally, PfJ2 uniquely contains both a J-domain and a thioredoxin-like domain, and we will explore how these domains contribute to PfJ2 function. By elucidating the role this essential chaperone plays in parasite biology, we will gain a better understanding of the mechanisms used by the parasite to survive in the RBC and cause disease.

217: Epigenetic reader complexes of Plasmodium falciparum

Hoeijmakers, Wieteke (Radboud University)*; Bartfai, Richard (Radboud University); Miao, Jun (Pennsylvania State University); Schmidt, Sabine (Bernhard Nocht Institute for Tropical Medicine); Shrestha, Sony (Pennsylvania State University); Venhuizen, Jeron (Radboud University); Henderson, Rob (Radboud University); Birnbaum, Jakob (Bernhard Nocht Institute for Tropical Medicine); Ghidelli, Sonja (Cellzome GmbH); Drewes, Gerard (Cellzome GmbH); Cui, Liwang (Pennsylvania State University); Stunnenberg, Hendrik (Radboud University); Spielmann, Tobias (Bernhard Nocht Institute for Tropical Medicine)*

Posttranslational modification of histone tails (PTMs) are key regulators of all DNA-associated processes including gene expression, replication and repair. In malaria parasites, epigenetic regulation has been associated with crucial adaptation processed such as antigenic variation, commitment to gametocytogenesis or drug resistance. While a plethora of PTMs has been identified in P. falciparum and genome-wide distribution of some of these marks show clear correlation to gene expression levels, we know almost nothing about how these epigenetic marks are interpreted by the parasite at the molecular level. Here we identified “reader” proteins recruited to specific epigenetic marks by histone peptide pull-down combined with a quantitative proteomic workflow. Using this approach, we established the association between 12 prominent epigenetic marks and 12 putative or novel reader proteins in blood stage parasites, highlighting, amongst others, the site-specific and acetylation-level dependent recruitment of all bromo-domain proteins.

Importantly, these pull-downs not only revealed the readers, but also associated protein complexes, which we validated and further characterized by epitope tagging and affinity purification of 12 candidate proteins. This way, we determined the composition of 5 core epigenetic complexes and revealed a remarkable connectivity between these complexes. Furthermore, we unveiled epigenetic mark-dependent changes in complex composition. For example, a SAGA-like complex is primarily recruited to H3K4me3 via a PhD-finger protein, but a slightly different complex can also bind to acetylated H4 via the bromodomain of GCN5. Notably, many of these complexes contain PfAP2 transcription factors indicating a widespread crosstalk between epigenetic and transcriptional regulation. Collectively, this study provides important insights to the recruitment of epigenetic reader complexes and opens new avenues towards the development of drugs targeting epigenetic reader proteins.

218: Post-translational Processing of a Secreted Putative Phosphatase in Toxoplasma gondii

Blakely, William (IU School of Medicine)

Toxoplasma gondii manipulates host cell systems including gene transcription, apoptosis cascades and innate immune recognition in order to maintain infection. This manipulation is accomplished in part by the secretion of parasite proteins both into the parasitophorous vacuole (PV) in which the parasite divides and into invaded host cells. Interestingly, many secreted parasite proteins known to modulate the host are kinases. Whether secreted phosphatases play a similar role remains largely unexplored. To address this knowledge gap, we identified 32 proteins predicted to have both a phosphatase domain and a signal sequence. Of interest is the protein IMC2A, which was previously identified as part of the inner membrane complex, but contains a signal sequence and predicted TEXEL motifs, protease cleavage sites typical of T. gondii secreted proteins. IMC2A has a predicted phosphatase domain homologous to purple acid phosphatases, a ubiquitous class of enzymes typically active under acidic conditions with a wide variety of protein and molecular substrates. By adding a C-terminal epitope tag to the protein, we have determined IMC2A is indeed processed and secreted into the PV.

Mutational analysis indicated the second of the two TEXEL sites is essential for processing. We have also added internal epitope tags, which allowed us to determine that both fragments resulting from processing are present in the PV. Immunoprecipitation showed IMC2A interacts with other well-known secreted factors. Current and future work is focused on measuring in vitro phosphatase activity of IMC2A and characterization of growth defects from IMC2A knockdown experiments.

219: An ER-resident calcium binding protein is required for egress and invasion of malaria parasites

Fierro, Manuel A. (The University of Georgia)*; Asady, Beejan (The University of Georgia); Brooks, Carrie (The University of Georgia); Moreno, Silvia (The University of Georgia); Muralidharan, Vasant (The University of Georgia)*

Plasmodium is the causative agent of malaria, a disease that continues to be a burden in large parts of the world. Calcium signaling has emerged as one of the major drivers of the life cycle of the deadly human malaria parasite, Plasmodium falciparum. Similar to other eukaryotic cells, the cytoplasmic levels of calcium are very low in P. falciparum and most of it is stored in intracellular calcium stores, such as the endoplasmic reticulum. To understand the role of the parasite ER in calcium signaling, we generated conditional mutants targeting a soluble ER-resident calcium binding protein (PfERC). Lifecycle analysis of the PfERC mutants revealed that egress from the RBC was affected and invasion was blocked upon knockdown. Our data show that proteolytic maturation of SUB1, AMA1, and MSP1 were all affected under knockdown conditions whereas the maturation of the rhoptry bulb protein, RAP1, was not. Ca2+ measurements using the cell-permeant Ca2+ indicator, Fluo-4AM suggest that the ER calcium storage is not affected by knockdown of PfERC, suggesting a role in calcium signaling. Ongoing experiments are testing this hypothesis. Our results show that PfERC is essential for parasite growth and is required for parasite egress and invasion.

220: Adaptation of Translation-Associated Machinery to the polyA Track-Rich Transcriptome in Plasmodium falciparum

Erath, Jessey (Washington University at St. Louis)*; Pavlovic-Djuranovic, Slavica (Washington University at St. Louis); Djuranovic, Sergej (Washington University at St. Louis)*

The complex life cycle and unique genome architecture of Plasmodium falciparum provide a plethora of unexplored biological questions; the study of which are necessitated by associated global health and economic impacts. Bioinformatic analyses have shown enrichment of known translational attenuators, polyadenosine (polyA) tracks, in Plasmodium spp. versus other organisms. A combination of AT-richness (~81%), an AAA codon bias (82%) and its in polylysine tracks significantly increases the number of these motifs in
P. falciparum, dichotomizing it from other sequenced organisms, hosts included. P. falciparum accurately translates polyA tracks by an as yet determined mechanism. This is of particular interest given the large number of polylysine track-containing genes (=60%), and their ostensible requirement to be efficiently translated for survival. While the biological relevance of polylysine tracks remains unknown, a connection between gene ontology and mechanisms of host immunity suggest a pathogenic function.To investigate contributing factors of the P. falciparum translation-associated machinery in intragenic polyA tolerance, RNA aptamer based pull-down methods have been adapted to isolate either mRNA- or ribosome-associated components. Both methods significantly enrich isolation of the target species by over three orders of magnitude allowing for the first direct examination of different mRNA and rRNA components in the parasite. Bioinformatic analyses and biochemical characterization implicate not only differences in the translational machinery composition given different mRNA substrates, but also in the outcomes of how such substrates are handled by the translation-associated quality control pathways. Mass spectrometry characterization of these differences is currently underway to discern the mechanisms by which the parasite tolerates its polyA track-rich transcriptome as well as investigation into how polylysine tracks subsequently contribute to pathogenesis.

221: H+-dependent inorganic phosphate uptake in Trypanosoma brucei is influenced by myo-inositol transporter

Heise, Norton (Universidade Federal do Rio de Janeiro)*; Russo-Abrahão, Thais (Universidade Federal do Rio de Janeiro); Koeller, Carolina (SUNY at Buffalo); Silva-Rito, Stephanie (Universidade Federal do Rio de Janeiro); Steinmann, Michael (University of Bern); Marins-Lucena, Thaissa (Universidade Federal do Rio de Janeiro); Alves-Bezerra, Michele (Universidade Federal do Rio de Janeiro); Lima-Giarola, Naíra (Universidade Federal do Rio de Janeiro); Francisco de-Paula, Iron (Universidade Federal do Rio de Janeiro); Gonzalez-Salgado, Amaia (University of Bern); Sigel, Erwin (University of Bern); Bütikofer, Peter (University of Bern); Calp Gondim, Kátia (Universidade Federal do Rio de Janeiro); Meyer-Fernandes, José Roberto (Universidade Federal do Rio de Janeiro)*

Trypanosoma brucei depends on exogenous inorganic phosphate (Pi) during its life cycle, but little is known about the transport of Pi in this organism. Here we showed that the transport of 32Pi across the plasma membrane of the parasite follows Michaelis-Menten kinetics with higher activity at acidic pH. Bloodstream forms presented 6-7 fold lower Pi transport in comparison to procyclics, that displayed an apparent K0.5 = 93 ± 8 µM and a Vmáx = 3.2 ± 0.1 nmol x min-1 x 10-7 cells. Incubation with the H+-ionophore FCCP, the K+ ionophore valinomycin, and the H+,K+-ATPase inhibitor SCH28080 inhibited the Pi transport in this parasite, an effect that was not observed with neither the H+-pump inhibitor bafilomycin A1 nor the pyrophosphate analog AMDP and the pyrophosphatase inhibitor IDP. Gene Tb11.02.3020, previously described to encode the parasite H+:myo-inositol transporter (TbHMIT), was hypothesized to be potentially involved in the H+:Pi cotransport because of its similarity with the Pho84 transporter described in S. cerevisiae and other trypanosomatids. Indeed, RNAi mediated knockdown reduced TbHMIT gene expression, compromised cell growth and decreased Pi transport by half. Pi transport was inhibited when parasites were incubated in the presence of concentrations of myo-inositol that were above 300 µM. However, when expressed in Xenopus laevis oocytes, two-electrode voltage clamp experiments provided direct electrophysiological evidence that the protein encoded by TbHMIT is definitely a myo-inositol transporter that may be only marginally affected by Pi. The results confirmed the presence of a Pi carrier in T. brucei, similar to the H+ -dependent Pi system described in S. cerevisiae and other trypanosomatids, but that is influenced by myo-inositol transporter.

222: Pharmacological disruption of ApiAP2 transcription factor function

Russell, Timothy J. (Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803)*; DeSilva, Erandi (Ohio State Drug Development Institute, Columbus, OH 43210); Bath, Jade (Department of Microbiology and Immunology, Columbia University, New York, NY 10027); Josling, Gabrielle (Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803); Painter, Heather (Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803); Panagiotou, Gianni (Leibniz Institute for Natural Product Research and Infection Biology, Hans Kroll Institute, Jena, Germany); Kirsch, Sierra (Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803); Fiddock, David (Department of Microbiology and Immunology, Columbia University, New York, NY 10027); Llinás, Manuel (Biochemistry and Molecular Biology Department, Huck Center for Malaria Research, Pennsylvania State University, University Park, PA 16803)*

Plasmodium parasites make use of fine-tuned transcriptional control to regulate the gene expression cascades that are necessary for their proliferation and transmission between hosts. Remarkably, they achieve this using a single family of 27 DNA binding proteins unique to Apicomplexans, the ApiAP2 proteins. To date, a handful of Plasmodium ApiAP2 proteins are known to be essential for the generation of developmental stages required for parasite transmission. ApiAP2 proteins are attractive drug targets because they have no homologs in humans due to their plant origin. Here, we report a growth arrest and transcriptional perturbation resulting from treatment with 'Compound G', a small molecule that was computationally docked within the dimerized AP2 domain of PF3D7_1466400. The interaction of Compound G with PF3D7_1466400 results in the disruption of binding to its cognate DNA motif in vitro. Addition of Compound G to blood stage parasites causes a state of developmental arrest that can be circumvented by removing drug prior to schizogony. This suggests a surprising divergence from the function of its P. berghei orthologue, AP2-Sp, which is not essential for blood stage proliferation. We are concurrently measuring the effect of disruption of PF3D7_1466400 on development by mislocalizing it away from the nucleus. Correlation of PF3D7_1466400 genome-wide occupancy by chromatin immunoprecipitation (ChIP-seq) to mRNA transcripts that are dysregulated in the presence of Compound G will define what targets of PF3D7_1466400 are affected during blood stage development. Analysis of resistant parasite lines selected at 1.5x the IC50 of Compound G is underway to ascertain a mechanism of action via whole genome sequencing.

223: Post-transcriptional regulation of the NT3 purine transporter in the Leishmania donovani purine stress response

Licon, Haley (Oregon Health and Science University)*; David, Larry (Oregon Health and Science University); Landfear, Scott (Oregon Health and Science University); Yates, Phillip (Oregon Health and Science University)*

Parasites must respond to changes in their hosts, yet in Leishmania species environmental adaptation is poorly understood. As Leishmania are auxotrophic for purines and have evolved a robust stress response that enables their long-term survival in the absence of these nutrients, we have used purine starvation as a paradigm to examine adaptive gene regulation in Leishmania donovani. One of the most dramatic changes observed in purine-starved Leishmania is directed at increasing purine salvage capacity through the upregulation of membrane purine transporters. Our recent efforts have focused on understanding how expression of these transporters is modulated in response to purine availability.Due to the polycistronic nature of transcription in kinetoplastids, Leishmania rely predominantly upon mechanisms affecting mRNA stability, translational efficiency, and protein half-life to modulate protein abundance. Using a series of integrating luciferase reporters, we have demonstrated that purine-responsive expression of the purine nucleobase transporter NT3 is regulated at all three of these levels. Over a 48-hour time course, NT3 mRNA abundance/translation increased continuously post-starvation. This upregulation was mediated by a 33-nt stem-loop in the NT3 mRNA 3' untranslated region that functions to repress NT3 expression under purine-replete conditions. Phosphoproteomic analysis revealed phosphorylation changes at several sites within a large intracellular loop of NT3. Replacement of this loop with that of NT4, a related but non-purine responsive transporter, yielded a chimeric transporter whose baseline expression was substantially higher than that of wildtype NT3. These data suggest that NT3 may be controlled post-translationally by elements within this region. Efforts to validate the regulatory function of the NT3 intracellular loop and to identify proteins interacting with the repressive mRNA stem-loop are ongoing.

224: CCCH zinc finger proteins regulate bradyzoite transition in Toxoplasma gondii.

Garfoot, Andrew (University of Wisconsin - Madison); *Knoll, Laura (University of Wisconsin - Madison)*

The success of Toxoplasma gondii can be attributed to its ability to form chronic infections in mammals. Unfortunately, the mechanisms to form the slow-growing chronic state (bradyzoite) is not well understood. To identify global parasite activity during chronic infection, our lab analyzed the Toxoplasma transcriptome of chronically infected mouse brain tissue. This analysis identified three highly expressed and chronically enriched genes (ZCH1-3) encoding for CCCH zinc finger proteins. CCCH proteins regulate translation by binding the poly-A tail of mRNA to target for degradation, in which many effected genes are important for developmental transition in plants, animals, and protozoans. Specifically, in Trypanosoma brucei, a CCCH gene alters the transition from the human bloodstream form to the insect form, suggesting Toxoplasma ZCH1-3 may be important for bradyzoite switching. To study their roles, ZCH1, 2, and 3 were knocked-out from the Toxoplasma strain ME49. ZCH1 and ZCH2 show dysregulation of bradyzoite markers during bradyzoite switch in vitro, and all three mutants have increased virulence in vivo during acute infection, suggesting the mutants are unable to switch efficiently from tachyzoite to bradyzoite. To understand the breadth of regulation these CCCH proteins have, I have developed a procedure to isolate cysts from chronically infected mouse brain tissue to perform a dual proteome-transcriptome analysis on the parasites. This analysis is currently being performed on wild-type Toxoplasma as proof of concept and will be repeated to analyze the mutants, allowing us to identify CCCH dependent translational control of genes. Understanding CCCH regulation will bring us closer to understanding how Toxoplasma transitions in the host and is able remain in the host for an extended time.

225: Investigating mutational pathways to resistance for clinically-relevant dihydroorotate dehydrogenase inhibitors

Mandt, Rebecca (Harvard T.H. Chan School of Public Health)*; Lafuente-Monasterio, Maria Jose (GlaxoSmithKline); Sakata-Kato, Tomoyo (Harvard T.H. Chan School of Public Health); Luth, Madeline (University of California San Diego); Segura, Delfina (GlaxoSmithKline); Pablos, Alba (GlaxoSmithKline); Ottilie, Sabine (University of California San Diego); Winzeler, Elizabeth (University of California San Diego); Gamo, F. Javier (GlaxoSmithKline); Wirth, Dyann (Harvard T.H. Chan School of Public Health); Lukens, Amanda (Harvard T.H. Chan School of Public Health)*

Resistance has emerged to every antimalarial therapy. In developing new drugs, we need to understand how resistance evolves so we can evaluate and extend the useful lifetime of new therapeutics. In this study, we focus on DSM265, a dihydroorotate dehydrogenase (DHODH) inhibitor which recently completed Phase II clinical trials. To investigate pathways to resistance for DSM265, we performed in vitro selections with DSM265 and the structurally-related DSM267. We additionally selected parasites in vivo using a humanized mouse model. In both systems, resistance arose rapidly.

Sequencing of resistant clones identified nine unique mutations in DHDOH: C276Y, F227L, F227Y, G181C, L531F, and V532G (selected in vitro) and C276F, G181D, and R265G (selected in vivo). Interestingly, the C276Y/F variants isolated from both in vitro and in vivo systems were also found in patients failing treatment with DSM265 during the Phase II trial. To determine whether clinical resistance to DSM265 results in reduced fitness, we performed in vitro competition assays with the DHODH:C276Y line and wildtype parent.

The C276Y mutation persisted in mixed culture at ~50% frequency over one month, demonstrating this mutation does not confer a significant fitness cost. We also assessed DHODH activity in a Seahorse bioenergetics assay, finding that the C276Y mutation has little impact on enzyme activity. Finally, we tested all DSM265-resistant lines for cross-resistance against two chemically-distinct DHODH inhibitors (Genz669178 and IDI6273). Most parasite lines, including DHODH:C276Y, exhibit hypersensitivity to at least one compound. This observation of “collateral sensitivity” has been previously observed by our group, and suggests that carefully-designed combination therapy could be used to target common mutational pathways to resistance for DSM265.

226: Externalized neutrophil components drive inflammatory pathogenesis in malaria

Lorenz Knackstedt[1], *Benjamin Mordmüller*[2], *Ulrike Abu-Abed*[1], *Arturo Zychlinsky*[1], *Borko Amulic*[1] *Max-Planck Institute for Infection Biology, Charitéplatz 1, 10117 Berlin, Germany* [2] *Institut für Tropenmedizin, Wilhelmstraße 27, 72074 Tübingen, Germany* [3] *University of Bristol, Tyndall Avenue, Bristol, BS8 1TH, UK*

Clinical symptoms of malaria only occur when the parasite undergoes asexual replication within the red blood cells of the host. Destruction of these cells and subsequent release of cytokines are responsible for the recurring fever cycles of mild malaria. The mechanism underlying tissue damage however, remain mostly elusive. The adhesion of infected red blood cells to the endothelial wall of the microvasculature in the affected organs is a necessary requirement and pathology is associated with the activation of specific immune cells residing within the blood stream. Severity of disease is also linked to extracellular accumulation of neutrophil proteins. Neutrophils are abundant white blood cells, known to readily deploy an arsenal of weaponry either by degranulation or by externalization of chromatin.
We report a direct causal relationship between the active inflammatory neutrophil cell death (NETosis) and the development of organ damage during a Plasmodium infection. We show that NETs are released in circulation, digested by extracellular DNase and thereby supply immune activation signals that drive inflammation. The systemic dissemination of these factors leads to the release of cytokines, emergency granulopoiesis and upregulation of cellular adhesion markers on endothelial cells thereby allowing for the binding of both infected red blood cells and immune cells to the microvasculature of specific organs. Furthermore we supply evidence, that repression of NETosis or inhibition of granulopoiesis abrogate these processes and present promising therapeutic strategies.

227: Splicing, translation and mRNA decay in Trypanosoma brucei

Clayton, Christine (ZMBH)*; Bajak, Kathrin (ZMBH); Bishola, Tania (ZMBH); Helbig, Claudia (ZMBH); Liu, Bin (ZMBH); Melo do Nascimento, Larissa (ZMBH); Terrao, Monica (ZMBH); Waithaka, Albina (ZMBH)*

We are studying various aspects of post-transcriptional regulation T. brucei. This includes: (a) the roles of the RNA-binding proteins ZC3H5 and ZC3H20-22; (b) the effect of splice signal changes on splicing and polyadenylation; (c) roles and interactions of possible splicing factors (including screening of a custom full-open-reading-frame yeast 2-hybrid library); (d) the effects of benzoxaboroles on mRNA processing and (e) the roles of mRNA-bound complexes containing PBP1. Selected aspects from one or more of these projects will be presented.

Author Index

E

L

N

Abel Steven
UC Riverside
sabel002@ucr.edu

Adame-Gallegos Jaime
Universidad Autónoma de Chihuahua
jadame@uach.mx

Afasizheva Inna
Boston University
innaaf@bu.edu

Alvarez Catalina
Instituto Gulbenkian de Ciencia
mcmeneses@igc.gulbenkian.pt

Ankarklev Johan
Weill-Cornell Medical College
johan_ankarklev@hotmail.com

Arranz-Solís David
UC Davis
darranz@ucdavis.edu

ASSISI CHRISTINA
NANYANG TECHNOLOGICAL UNIVERSITY
christin007@e.ntu.edu.sg

Ayaz Muhammad Mazhar
Bahauddin Zakariya University
mazharayaz@bzu.edu.pk

Balasubramaniyam Arasu
NIAID (NIH)
arasu.balasubramaniyam@nih.gov

Baptista Carlos
Department of Microbiology and Immunology, University at Buffalo
cgbaptis@buffalo.edu

Barbieri Daniela
INSERM
daniela.barbieri@inserm.fr

Basson Travis
Swiss TPH, University of Basel
travis.basson@swisstph.ch

Baumgarten Sebastian
Biology of Host-Parasite Interactions Unit, Institut Pasteur Paris
sebastian.baumgarten@pasteur.fr

Beck Josh
Iowa State University
joshryanbeck@gmail.com

Berná Luisa
Institut Pasteur de Montevideo
lberna@pasteur.edu.uy

Bettadapur Akhila
University of California, Davis
abett@ucdavis.edu

Bi Feifei
China agricultural university
475080602@qq.com

Bishnoi Ritika
Temple University School of Medicene, Philadelphia
ritikabishnoi86@gmail.com

Blakely William
IU School of Medicine
wblakely@iupui.edu

Bopp Selina
Harvard T.H.Chan School of Public Health
sbopp@hsph.harvard.edu

Bowden Gregory
Washington State University
gregory.bowden@wsu.edu

Brancucci Nicolas
SwissTPH, University of Basel
nicolas.brancucci@swisstph.ch

Brown Kevin
Washington University in St. Louis
brown.wustl@gmail.com

Bryant Jessica
Institut Pasteur
jessica.bryant@pasteur.fr

Campelo-Morillo Riward
Weill Cornell Medicine
rac2032@med.cornell.edu

Carmeille Romain
University of Connecticut
romain.carmeille@uconn.edu

Casas-Sanchez Aitor
Liverpool School of Tropical Medicine
aitor.casas-sanchez@lstmed.ac.uk

Chalapareddy Suresh
NIH
chalapareddyusa@gmail.com

Chaudhuri Minu
Meharry Medical College
mchaudhuri@mmc.edu

Chauvet Margaux
UMR216 IRD-Universite Paris Descartes
margauxchauvet@hotmail.fr

Cipriano Michael
University of Georgia, Athens
mcipriano@gmail.com

Clements Rebecca
Harvard University
rebecca_clements@g.harvard.edu

Colon Beatrice
University of Georgia
bc74328@uga.edu

Cooper Roland
Dominican University of California
roland.cooper@dominican.edu

Cruz Angela
University of Sao Paulo
akcruz@fmrp.usp.br

Cunningham Deirdre
The Francis Crick Institute
deirdre.cunningham@crick.ac.uk

Das Anish
Rutgers - New Jersey Medical School
dasak@njms.rutgers.edu

Davies Heledd
Francis Crick Institute
heleddd@gmail.com

Del Rosario Mario
University of Glasgow - Wellcome Centre for Molecular Parasitology
m.del-rosario-minina.1@research.gla.ac.uk

Dey Vishakha
IIT Bombay
vishakhadey@gmail.com

Doggett Joseph
Oregon Health and Science University Portland VA Medical Center
doggettj@ohsu.edu

Dong Gang
Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Austria
gang.dong@meduniwien.ac.at

Dou Zhicheng
Clemson University
zdou@clemson.edu

Driskell I
Washington State University
iwona.driskell@wsu.edu

Dupuy Florian
inserm U1016 / CNRS 8104
florian.dupuy@inserm.fr

Dvorin Jeffrey
Harvard Medical School / Boston Children's Hospital
jeffrey.dvorin@childrens.harvard.edu

English Elizabeth
University of Pennsylvania
elien@vet.upenn.edu

Erath Jessey
Washington University at St. Louis
jlerath@wustl.edu

Espino Tanya
University of Utah
tanya.espino@biochem.utah.edu

Evans Rachel
Johns Hopkins Bloomberg School of Public Health
revans46@jhmi.edu

Fagbami Lola
Harvard University
fagbami@g.harvard.edu

Feeney Shea, E.
University of California, Davis
sefeeney@ucdavis.edu

Filarsky Michael
Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland
michael.filarsky@unibas.ch

Florentin Anat
University of Georgia
anat@uga.edu

Funkhouser-Jones Lisa
Washington University School of Medicine
l.funkhouser-jones@wustl.edu

Garrison Paige
Univeristy at Buffalo
pd32@buffalo.edu

Gibson Alexis
University of Pennsylvania
algi@upenn.edu

Gold Daniel
St. Edward's University
dgold@stedwards.edu

Grajeda Brian
University of Texas at El Paso
bgrajeda16@gmail.com

Gubbels Marc-Jan
Boston College
gubbelsj@bc.edu

Haidar Malak
KAUST, BESE DIVISION
malak.haidar.1@kaust.edu.sa

Haldar Kasturi
University of Notre Dame
khaldar@nd.edu

Harb Omar

oharb@upenn.edu

Hart Kevin
The Pennsylvania State University
kevin.hart@psu.edu

HE XIAO
NIH/NIAID
xiao.he@nih.gov

Heise Norton
Universidade Federal do Rio de Janeiro
nheise@biof.ufrj.br

Henrici Ryan
London School of Hygiene & Tropical Medicine
ryan.henrici@lshtm.ac.uk

Hickman Mark
University of Pennsylvania

Hovel-Miner Galadriel
The George Washington University
ghovel_miner@gwu.edu

Huet Diego
Whitehead Institute
dhuet@wi.mit.edu

Iyengar Kalpana
Departments of Chemistry, and of Biochemistry and Cellular and Molecular Biology, Georgetown University, 37th and O St. NW, Washington, DC.
ki81@georgetown.edu

Jankowska-Döllken Monika
Heidelberg University Hospital
monika.jankowska@med.uni-heidelberg.de

Jimenez Veronica
California State University Fullerton
vjimenezor@gmail.com

Kariuki Silvia
KEMRI-Wellcome Trust Research Programme
snkariuki@kemri-wellcome.org

KE HUILING
UNIVERSITY OF CAMBRIDGE
hk434@cam.ac.uk

Khair Nima Maisha
University of Notre Dame
mnima@nd.edu

Kissinger Jessica
University of Georgia
jkissing@uga.edu

Klinger Christen
University of Alberta
cklinger@ualberta.ca

Ko Daphne
CUNY Hunter College
daphneko524@gmail.com

Koeller Carolina
University at Buffalo
ckoeller@buffalo.edu

Kooij Taco
Radboud University Medical Centre, Nijmegen, Netherlands
taco.kooij@radboudumc.nl

Krishnamurthy Shruthi
University of California, Davis
skrishnamurthy@ucdavis.edu

Kuchipudi Arunakar
Humboldt University Berlin
arunakar.kuchipudi@hu-berlin.de

Kumar Pradeep
International Centre For Genetic Engineering and Biotechnology New Delhi
pradeep.sheokand6@gmail.com

Lacombe Alice
Wellcome Centre for Molecular Parasitology, University of Glasgow
a.lacombe.1@research.gla.ac.uk

Lane Kristin
NIAID/NIH
kristin.lane@nih.gov

Lebrun Maryse
CNRS-Université Montpellier 2
mylebrun@univ-montp2.fr

Lee Penny
CUNY Hunter College
pensqlee@gmail.com

Li Yiran
University of Georgia
yl22225@uga.edu

Lindner Scott
Pennsylvania State University
scott.lindner@psu.edu

Liu Ying
Nanyang Technological University
yliu037@e.ntu.edu.sg

Llorà-Batlle Oriol
ISGlobal
oriol.llora@isglobal.org

Lorenzon Lucas
University of São Paulo
lucas_lorenzon@hotmail.com

Lunghi Matteo
University of Geneva
matteo.lunghi@unige.ch

Magistrado-Coxen Pamela
Boston University
pmagist@bu.edu

Malvezzi Amaranta
Federal University of São Paulo
amarantamalvezzi@gmail.com

MANNA DIPAK
Stanford University
dmanna@stanford.edu

Markéta Petru
Department of Parasitology, Faculty of Science, Charles University, Prumyslová 595, Vestec, 252 42, Czech Republic
petrum@natur.cuni.cz

Martorelli Di Genova Bruno
University of Wisconsin - Madison
martorellidi@wisc.edu

Marzluf Tanja
Centre of Infectious Diseases, Parasitology Department
t.marzluf@gmail.com

Matthews Holly
Keele University
h.matthews@keele.ac.uk

Mbekeani Alison
Durham University
a.j.mbekeani@durham.ac.uk

McGuffie Bryan
Broad Institute
mcguffie@broadinstitute.org

McMahan Timothy
University of Louisiana at Monroe
mcmahats@warhawks.ulm.edu

Meissner Markus
University of Glasgow
markus.meissner@glasgow.ac.uk

Mesitov Mikhail
Department of Molecular and Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA
mmesitov@bu.edu

Miller Hannah W.
University of California, Davis
hwmiller@ucdavis.edu

Mixon Amanda
University of Utah
amanda.mixon@biochem.utah.edu

MOITRA SAMRAT
Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409
samrat.moitra@ttu.edu

Moliner Cubel Sonia
GSK
sonia.x.moliner@gsk.com

Moore Sheridan Christine
University of California, San Francisco
christine.sheridan@ucsf.edu

Motyckova Alzbeta
Department of Parasitology, BIOCEV, Faculty of Science, Charles University in Prague, Czech Republic
motyckova.alzbeta@gmail.com

Mukherjee Debanjan
Instituto de Medicina Molecular, Faculadade de Medicina de Lisboa
debanjan507@gmail.com

Mukhopadhyay Debanjan
University of California, Davis
dmukhopadhyay@ucdavis.edu

Munro Justin
Chemistry Department, Center for Malaria Research (CMaR), The Pennsylvania State University
jtm5518@psu.edu

Murphy Robert
University of Kentucky
rdmu223@uky.edu

Neveu Gaëlle
INSERM
gaelle.neveu@inserm.fr

Ning Yu
Texas Tech University
yu.ning@ttu.edu

Olafsson Einar
Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute
einar.olafsson@su.se

Orozco Daniel
University of Louisiana at Monroe
orozcodr@warhawks.ulm.edu

Paige Andrew
National Institute of Health
andrew.paige15@ncf.edu

Pala Zarna
National Institutes of Health
zarnapala@gmail.com

Palmer Margot
University of Georgia
shajduk@uga.edu

Pant Akansha
National Institute of Malaria Research
akansha@mrcindia.org

Paredes Santos Tatiana
UCDavis
tcparedessantos@ucdavis.edu

Paul Kimberly
Clemson University
kpaul@clemson.edu

Peirasmaki Dimitra
Uppsala University
dimitra.peirasmaki@icm.uu.se

Pierre Louis Edwin
University of Georgia Athens
epl1@uga.edu

Pivovarova Yulia
Medical University of Vienna
yulia.pivovarova@univie.ac.at

Potapenko Evgeniy
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia
epotapenko@uga.edu

Prigge Sean
Johns Hopkins School of Public Health
sprigge@jhsph.edu

Probst Alexandra
Center for Infectious Disease Research
alli.probst@cidresearch.org

Quadiri Afshana
National Institute of Immunology
qafshan.aq@gmail.com

RAJ DIPAK
Brown University
dipak_raj@brown.edu

Ralston Katherine
University of California, Davis
ksralston@ucdavis.edu

Rangel Gabriel
Harvard University
grangel@fas.harvard.edu

Reese Michael
UT Southwestern Medical Center
michael.reese@utsouthwestern.edu

Ridewood Sophie
The Francis Crick Institute
sophie.ridewood@crick.ac.uk

Roepe Paul
Georgetown University
roepep@georgetown.edu

Rudlaff Rachel M.
Harvard Medical School
rudlaff@g.harvard.edu

sabatini robert
university of georgia
rsabatini@bmb.uga.edu

Saggu Gagandeep
LMVR, NIAID, National Institutes of Health
gagan.saggu@nih.gov

Sam-Yellowe Tobili
Cleveland State University
t.sam-yellowe@csuohio.edu

Sayeed Abeer
Johns Hopkins Bloomberg School of Public Health
asayeed1@jhmi.edu

Schureck Marc
NIAID
marc.schureck@nih.gov

Shao Jinfeng
NIAID, NIH, Bethesda, MD, USA
jinfeng.shao@nih.gov

Shears Melanie
Johns Hopkins University
malariamel@gmail.com

Sinai Anthony
University of Kentucky College of Medicine
sinai@uky.edu

SINHA AMEYA
NANYANG TECHNOLOGICAL UNIVERSITY
ameya.sinha@gmail.com

Sollelis Lauriane
Welcome Center for Molecular Parasitology
lauriane.sollelis@glasgow.ac.uk

Srivastava Ankita
Department of Genetics and Genome Sciences, UConn Health, Farmington, CT
ankita26@gmail.com

Sterkers Yvon
University Montpellier 1
yvon.sterkers@umontpellier.fr

Stevens Grant
University of Toronto
grant.stevens@mail.utoronto.ca

Stuart Ken
Center for Infectious Disease Research
ken.stuart@seattlebiomed.org

Sullivan David
Johns Hopkins Bloomberg School of Public Health
dsulliv7@jhmi.edu

Suo Xun
China Agricultural University
suoxun@cau.edu.cn

Suvorova Elena
University of South Florida
essuvorova@gmail.com

Tagoe Daniel
Boston College
tagoe@bc.edu

Tanaka Takeshi
Kagawa University
tanakatq@med.kagawa-u.ac.jp

Thiam Laty
West African Centre for Cell Biology of Infectious Pathogens
latygaye.thiam@ucad.edu.sn

Tintó-Font Elisabet
ISGlobal
elisabet.tinto@isglobal.org

Tripathi Jaishree
Nanyang Technological University
tjaishree@ntu.edu.sg

Usui Miho
Henry M Jackson Foundation/USUHS
miho.usui.ctr@usuhs.edu

Van Biljon Riette
University of Pretoria
1riet21@gmail.com

Vandomme Audrey
Francis CRICK Institute
audrey.vandomme@crick.ac.uk

Verdi Joey
Hunter College, City University of New York
joseph.verdi05@gmail.com

Vinayak Sumiti
University of Illinois- Urbana Champaign
sumiti@illinois.edu

Voleman Lubos
Department of Parasitology, Charles University
lubos.voleman@gmail.com

Wahlgren Mats
Karolinska Institutet, and Modus Therapeutics AB,Stockholm
mats.wahlgren@ki.se

Wang Chaoyue
China agricultural university
906513329@qq.com

Wang Yifan
UC Davis
yifwang@ucdavis.edu

Warrenfeltz Susanne
University of Georgia
swfeltz@uga.edu

Wells Michael
Johns Hopkins University
mwells24@jhmi.edu

White Michael
University of South Florida
mwhite.usf@gmail.com

Wilson Sarah
University of Wisconsin Madison
skwilson3@wisc.edu

Yagoubat Akila
UMR MIVEGEC (CNRS 5290 - IRD 224 - Universités Montpellier 1 et 2)
akila.yagoubat@etu.umontpellier.fr

Yang Jing
Yale University School of Medicine
jing.yang@yale.edu

Zhang Kai
Texas Tech University
kai.zhang@ttu.edu