SPPIRIT
Scottish Parasitology Partnership in Research, Innovation and Training

SPPIRIT ECR SYMPOSIUM

25 OCTOBER 2024

University of the West of Scotland,
Lanarkshire Campus

@SPPIRITNetwork

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If you are interested in joining the SPPIRIT committee, contact us at sppirit.network@gmail.com or speak to one of the organising committee.

Acknowledgements

The SPPIRIT Organising Committee wishes to thank the following organisations for their kind financial contributions.

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Programme

09:00 - 09:50 Coffee and Registration

09:50 - 10:00 Welcome and Introduction

10:00 - 11:10 Science talks session 1

10:00 - 10:30 Keynote lecture 1: Beric Gilbert (UWS): "Ecosystem health under siege: Can ectoparasites add to understanding environmental changes?"

10:30 – 10:40 Jennifer McIntyre (UOG): "The genetic basis of anthelmintic resistance in Teladorsagia circumcincta: findings using a chromosomal assembly."

10:40 – 10:50 Kyle Cunningham (UOG): "A Family Of Helminth-Derived Tgf-β Mimics Target Tissue-Resident Macrophages And Granulocytes In The Peritoneal Cavity."

10:50 – 11:00 Martha Kivecu (UOE): "The Origin and Evolution of Plasmodium falciparum."

11:00 – 11:10 Matt Gibbins (UOG): "Imaging Plasmodium gametocytes in the skin."

11:10 - 12:30 Poster Session 1

Coffee break and poster session for odd numbers (1-21).

12:30 - 13:30 Lunch

Lunch provided by SPPIRIT. Please use this time to swap session 1 posters with session 2 posters.

13:30 – 14:30 Networking workshops

Career development session featuring short talks from professionals working in industry and healthcare followed by a question and answer session.

14:30 – 15:50 Poster Session 2

Coffee break and poster session for even numbers (2-20).

15:50 – 17:10 Science talks session 2

15:50 – 16:00 Laurine Brouck (UOD): "Two peas in a pod? The roles of REL1 and REL2 in uridine insertion/deletion RNA editing."

16:00 – 16:10 Lucas Pagura (UOG): "Copper uptake in the intracellular parasite Toxoplasma gondii."

16:10 – 16:20 Petra Schneider (UOE): "Are regular mealtimes more important for artemisinin resistant parasites?"

16:20 – 16:30 Shkiha Shkiha (UOG): "Adaptation to disruption: evolutionary impact of extreme rRNA fragmentation in Toxoplasma mitoribosome."

16:30 – 16:40 Simone Altmann (UOD): "Deep mutational resistance profiling for anti-trypanosomal proteasome inhibitors."

16:40 – 17:10 Keynote lecture 2: Abhinay Ramprasad (UOG): "Rhomboid proteases in the malaria parasite: The essentials"

17:10 – 17:20 Closing remarks and feedback.

17:20 – 18:30 SPPIRIT mixer.

18:30 Buses depart.

Poster session 1 – 11:10 – 11:30

1. Dancing out of step: The adaptability of Anopheles stephensi rhythms and their impact on malaria development.
Aidan J. O'Donnell, University of Edinburgh.

3. FIT plays a role in iron acquisition in Toxoplasma gondii
Dana Aghabi, University of Glasgow

5. A role for VSG mRNA in driving allelic competition
Douglas O Escrivani, University of Dundee

7. Interrogating genetic determinants of P. falciparum vector transmission
Duangkamon Loesbanluechai, University of Glasgow

9. Quinoxaline-Based Anti-Schistosomal Compounds Have Potent Anti-Plasmodial Activity
Mukul Rawat, University of Dundee

11. Resistance-associated mutations in the target of acoziborole - trypanosome cleavage and polyadenylation specificity factor 3
Melanie C. Ridgway, University of Dundee

13. How do biological rhythms impact parasite infection and transmission in wild wood mice?
Aniela M Dexter, University of Edinburgh

15. Chemical pulldown of anti-malarial TCMDC-135051 identifies unexpected target in Cryptosporidium
Grant M J Hall, University of Dundee

17. The relationship between expelled eggs, morbidity and age: Implications for current S. mansoni elimination policies
Rivka M. Lim, University of Edinburgh

19. Targeting epigenetic regulators in Plasmodium
Jed Hawes, University of Dundee

21. Permeability and morphological dynamics during Plasmodium falciparum gametocyte maturation.
Jahiro Gómez, Institute for Scientific Research and High Technology Services, Panama

23. Iron deprivation flips a metabolic switch towards anaerobic metabolism in Toxoplasma gondii
Jack Hanna, University of Glasgow

Poster session 2 – 14:30 – 15:50

2. Developing a Cre & DiCre conditional gene expression system to investigate essential C. parvum genes
Kai Lynn Wong, University of Dundee

4. Unravelling the Molecular Mechanisms of Toxoplasma gondii Complex II
Kiera Douglas, University of Glasgow

6. Let's talk about Secs: a novel anti-leishmanial drug target
Jack Duggan, University of Dundee

8. Cryptosporidium parvum infection is much more widespread throughout the calf GI than previously assumed.
Peyton Goddard, University of Dundee

10. Functional control via redox regulation and potential inhibition of the divergent Toxoplasma gondii succinate dehydrogenase (mETC complex II)
Mariana F. Silva, University of Glasgow

12. Direct demonstration that specific histone H4 tail lysines impact chromatin-based mechanisms in trypanosomes
Markéta Novotná, University of Dundee

14. Exploring mutator phenotypes to facilitate the understanding of Plasmodium falciparum antimalarial drug resistance
Edem Adika, University of Dundee

16. Do rhythmic interactions between mosquitoes and their microbiota influence malaria transmission?
Naomi Riithi, University of Edinburgh

18. Antimicrobial resistance and Contaminated soils
Sarah I. Bassey, University of the West of Scotland

20. Molecular Characterization of Opisthorchis viverrini TGF-beta Homologue and Role in Host-Parasite Interactions
Nuttanan Hongsrichan, University of Glasgow

22. Characterising the Oocyst Wall Proteins at the Cryptosporidium suture
Sarah Stevens, University of Dundee

24. ATR, a DNA damage kinase, modulates DNA replication timing by ensure the progress of the process over potential genomic replicative stress regions in Leishmania major.
Gabriel Almeida da Silva, University of Glasgow

Oral Presentations

10:30 – 10:40

The genetic basis of anthelmintic resistance in Teladorsagia circumcincta: findings using a chromosomal assembly

McIntyre, J. [1], Morrison, A. [2], Price, D. [2], Holroyd, N. [3], Bull, K. [4], Rose-Vineer, H. [4,5], Glover, M.J. [6], Morgan, N. [4,7], Nisbet, A. [2], Sargison, N. [8], Bartley, D. [2], Devaney, E. [1], Laing, R. [1], Doyle, S.R. [3]

1. School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow; 2. Moredun Research Institute, Penicuik; 3. Wellcome Sanger Institute, Hinxton; 4. Veterinary Parasitology and Ecology Group, University of Bristol; 5. Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool; 6. Torch Farm & Equine Ltd., Veterinary Surgeons, South Molton; 7. School of Biological Sciences, Queen's University Belfast; 8. Royal (Dick) School of Veterinary Studies, University of Edinburgh.

The sheep nematode Teladorsagia circumcincta is capable of rapidly developing anthelmintic resistance, with established resistant populations commonplace and multi-drug resistant populations steadily increasing. Identification of genetic mutations underlying resistance is complicated by a large genome (573 Mb) and large, highly heterogenous populations, but small amounts of DNA per worm. By whole genome sequencing pools of worms and using ddRAD-Seq on individuals, we have found distinct loci associated with benzimidazole, levamisole and macrocyclic lactone resistance. To do this we used field samples collected pre- and post-treatment, UK lab isolates and data archived from a genetic cross by Choi et al., (2017) between two NZ isolates - one sensitive and one triple-resistant. I will briefly describe how we completed the semi-manual curation of a chromosomal genome assembly for this parasite, and present the various loci associated with resistance to each anthelmintic, highlighting which experiments worked particularly well, and which could only be interpreted in light of the others.

10:40 – 10:50

A family of helminth-derived TGF-β mimics target tissue-resident macrophages and granulocytes in the peritoneal cavity.

Kyle T. Cunningham [1], Natalia Wąsowska [1], Shashi P. Singh [1,2], Maarten van Dinther [3], Tiffany Campion [1], Ananya Mukundan [4], Andrew P. Hinck [4], Peter ten Dijke [3], and Rick M. Maizels [1]

1. School of Infection and Immunity, University of Glasgow UK; 2. Department of Biological Sciences, BITS Pilani, Rajasthan, India; 3. Department of Cell and Chemical Biology, Leiden University Medical Center, The Netherlands; 4. Department of Structural Biology, University of Pittsburgh, USA.

Helminths have evolved sophisticated methods for manipulating the host immune response for long-term survival, primaril through the secretion of immunomodulatory proteins. Studies on the secreted products of Heligmosomoides polygyrus have identified a novel TGF-β mimic (TGM1). In vitro, TGM1 induces the differentiation of murine and human T regulatory (Treg) cells, while in vivo TGM1 ameliorates airway inflammation and colitis. Other TGMs are now known to bind specific cell types due to co-receptor ligation. Thus, TGM1 binds not only TGF-β receptors, but also CD44, a cell surface marker on effector T cells and macrophages. Therefore, H. polygyrus has evolved to secrete TGM1 to act preferentially on cells which specifically co-express TGF-β receptors and CD44. Meanwhile, TGM4 targets macrophages and induces an anti-inflammatory state, suppressing secretion of pro-inflammatory cytokines in response to LPS co-stimulation. This anti-inflammatory activation of macrophages induces markedly different signalling pathways due to this unique co-receptor ligation. In vivo, however, intraperitoneal administration results in the loss of tissue-resident peritoneal macrophages and eosinophils, with concomitant infiltration of anti-inflammatory monocytes for both TGM1 and TGM4. Understanding these variances will provide key insights to helminth immunomodulation, including the identification of latent co-receptors that may provide unique targets for drug discovery.

10:50 – 11:00

The Origin and Evolution of Plasmodium falciparum

Martha Kivecu, Brian Omondi, Reuben Nowell, Paul Sharp

Institute of Ecology and Evolution, University of Edinburgh; Institute of Immunology and Infection Research, University of Edinburgh.

The malignant human malaria parasite Plasmodium falciparum evolved from the zoonotic transmission of a gorilla parasite, Plasmodium praefalciparum. However, the exact mechanism and timing of this event remain a matter of debate. Some have suggested that P. falciparum arose around 60,000 years ago, while others have suggested that it occurred after the origin of agriculture, less than 10,000 years ago.

In both humans and a bacterial pathogen, Helicobacter pylori, it has been reported that levels of genetic diversity decrease with distance from the area of origin of modern humans in East Africa. In an analysis of the genetic diversity using two housekeeping genes from 519 samples, Tanabe et al. (2010), found that the genetic diversity of P. falciparum also decreased with distance from the eastern part of Central Africa. They interpreted this as evidence that P. falciparum emerged from Africa at the same time as humans, around 60,000 years ago.

Now, thousands of P. falciparum whole genome sequences are available. This study aims to determine the geographical distribution of genome-wide genetic diversity and its implications for the origin of P. falciparum. Genome-wide genetic diversity was calculated for a total of 10, 333 sequences from 88 study sites in 33 countries. As expected, it was found that the genome-wide nucleotide diversity was higher in African sites (0.050%) as compared to other regions of the world (0.038% in Oceania, 0.035% in Asia, 0.031% in S America).

For the 46 study sites across Africa, the 'least-cost' geographical distance from each of 2,000 uniformly spaced potential sites of origin was calculated, and the Spearman correlation between genome-wide genetic diversity and least-cost geographical distance was calculated. The highest negative correlation was obtained for locations in the Western part of central Africa, close to the range of Western lowland gorillas (Gorilla gorilla) that harbour P. praefalciparum, i.e., the genetic diversity of P. falciparum declines with distance from where western gorillas are found. Since this correlation is expected to be disrupted by demographic factors over time, this finding is consistent with a very recent origin of P. falciparum.

11:00 – 11:10

Imaging Plasmodium gametocytes in the skin

Matthew P Gibbins [1], Thomas Purnell [1], Leo Carlin [2], Matthias Marti [1,3]

1. University of Glasgow; 2. CRUK Scotland Institute; 3. University of Zurich

The sexual stages of Plasmodium malaria parasites, the gametocytes, need to be uptaken by mosquitoes to sexually reproduce and produce sporozoites, to continue the parasite's life cycle in the vertebrate host. However, there are individuals with malaria that have a very low burden of parasites, but yet are still able to infect mosquitoes at a rate higher than you would expect. In order to explain this phenomenon, we are investigating where gametocytes are in the skin using a variety of different techniques. In order to visualise the behaviour of gametocytes in the skin, we have been developing an intravital microscopy platform using an inverted confocal microscope and specialised stage. After performing surgery on Plasmodium berghei infected mice to generate a skin flap, we are able to image the fluorophore-expressing parasites as they move in the vasculature and tissue of the skin. In this way, we will be able to start quantifying the phenotypes that we see and then determine how these change when a mosquito feeds on the skin.

15:50 – 16:00

Two peas in a pod? The roles of REL1 and REL2 in uridine insertion/deletion RNA editing

Laurine Brouck [1], Zandile Nare [1], James Smith [2], Eva Gluenz [2], Atlanta Cook [3], Achim Schnaufer [1]

1. Institute for Immunology and Infection Research, University of Edinburgh, UK; 2. Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; 3 Institute of Quantitative Biology, Biochemistry and Biotechnology, University of Edinburgh, UK.

Uridine insertion/deletion RNA editing is essential for mitochondrial gene expression in kinetoplastids. Two paralogous RNA editing ligases, REL1 and REL2, perform the final step of each editing event. While REL1 is essential for Trypanosoma brucei survival, REL2 knockdown by RNAi did not produce any detectable phenotype. This is unexpected since REL1 and REL2 are active in vitro and their genes are under strong purifying selection, implying important functions for both ligases.

Through structure-function and transcriptomics analyses, we investigated the respective roles of REL1 and REL2 in RNA editing. A comparison of substrate requirements using recombinant proteins showed that REL1 is more tolerant than REL2 towards nucleotide mismatches in RNA substrates, likely due to its more flexible interdomain linker. Moreover, the essentiality of REL1 and dispensability of REL2 appear to be conserved among kinetoplastids, as they were confirmed in Leishmania mexicana via CRISPR-based knockouts. The editing patterns in REL2 null mutants were examined in preliminary RNA-Seq analyses, suggesting a proof-reading function for this ligase.

In summary, our data support the functional divergence of REL paralogs and suggest a structural basis for differences in substrate specificities and functions. These findings advance our mechanistic understanding of the intricate RNA editing process in kinetoplastids.

16:00 – 16:10

Copper uptake in the intracellular parasite Toxoplasma gondii

Lucas Pagura [1], Capucine Marlet [1,2], and Clare R. Harding [1]

1. School of Infection and Immunity, University of Glasgow, Glasgow, UK; 2. Institut Universitaire de Technologie (IUT), d'Evreux, Université de Rouen, Evreux, France.

Toxoplasma gondii is an obligate intracellular parasite that can infect almost all warm-blooded animals. Toxoplasma invades any nucleated cell type and replicates inside of a parasitophorous vacuole, before bursting the host and releasing more parasites, which causes acute Toxoplasmosis and can cause severe disease in immunocompromised or pregnant people. T. gondii relies on its host for nutrients, including copper which is essential as a cofactor in respiration as part of the cytochrome c complex (complex IV). However, how these parasites take up and respond to changing copper availability while causing infection and disease is not understood. We tested T. gondii sensitivity to changes in copper availability and used RNA-Seq to investigate the impact of copper excess and deprivation at a transcriptomic level. Our results shows that surprisingly a homolog of the copper transporter Ctr1 is upregulated under copper excess. We deleted Ctr1 and showed that deletion has a deleterious impact on the parasite fitness, inhibiting the lytic cycle and intracellular replication. Interestingly, some of these phenotypes can be rescue by exogenous copper supplementation of the culture media, suggesting that copper can be incorporated into the parasite through other, lower affinity, transporters. We found out that sensitivity to copper is reduced when Ctr1 gene is absent. Also, deletion of Ctr1 suppress complex IV activity and inhibited mitochondrial respiration, as observed by Seahorse assay. These data provide the first evidence of the relevance of copper for vital cellular processes in Toxoplasma gondii.

16:10 – 16:20

Are regular mealtimes more important for artemisinin resistant parasites?

Petra Schneider, Aidan J. O'Donnel, Sarah E. Reece.

Inst. of Ecology and Evolution, University of Edinburgh

The spread of malaria parasites resistant to the most common antimalarial drugs, the artemisinins, is of increasing concern. Resistant parasites inhibit both activation of the drug and its efficacy via less efficient haemoglobin digestion. However, haemoglobin digestion provides an important source of nutrients, especially amino acids, to replicating parasites. Acquiring nutrients is so important to malaria parasites that they time replication to synchronise with the host's daily feeding rhythm. If parasites cannot replicate on time their productivity and infectivity to the mosquito vector both drop. We predict that impaired haemoglobin digestion by drug resistant parasites exacerbates the importance of replicating at the right time of day. We used the rodent malaria, Plasmodium chabaudi to test this prediction, by comparing the parasite dynamics of an artemisinin sensitive genotype to an isogenic resistant genotype within infections where parasites were either aligned, or misaligned with the recipient host's rhythms. Our results show that artemisinin resistant parasites suffer more from misalignment to host feeding rhythms. Our findings suggest the ongoing shifts in the time-of-day malaria transmission occurs - by mosquitoes avoiding bednets - could help curb the spread of drug resistant genotypes.

16:20 – 16:30

Adaptation to disruption: evolutionary impact of extreme rRNA fragmentation in Toxoplasma mitoribosome

Shikha Shikha [1], Victor Tobiasson [2], Mariana F Silva [1], Jana Ovciarikova [1], Dario Beraldi [1], Alexander Mühleip [3] and Lilach Sheiner [1].

1. Centre for Parasitology, University of Glasgow; 2. National Center for Biotechnology Information: Bethesda, Maryland; 3. Institute of Biotechnology, University of Helsinki.

Mitochondrial ribosomes (mitoribosomes) are required for mitochondrial biogenesis, and therefore survival in nearly all eukaryotes. Despite sharing common ancestry, mitoribosomes have evolved remarkably divergent features in different eukaryotic lineages. Mitoribosomes in Apicomplexan parasites such as Plasmodium and Toxoplasma are expected to be unique due to the predicted extreme fragmentation and reduction of mitochondrial rRNAs.

Using CryoEM and various biochemical tools, we report high-resolution structure of the 4.3 MDa mitoribosome from the model Apicomplexa, Toxoplasma gondii, containing an astonishing 53 ribosomal RNA (rRNA) fragments, the most fragmented rRNA known in nature. We identify novel features that mitigate this extreme fragmentation, including incorporation of polyA tails as integral parts of the mitoribosome; recruitment of proteins mediating rRNA fragment assembly; and extensive use of small peptides to promote rRNA fragment contacts. Additionally, we show evidence for essentiality of mitochondrial polyA tail addition for mitoribosomal stability through knockdown of a mitochondrial PolyA polymerase. Interestingly, Toxoplasma mitoribosome also contains several transcription factor-like proteins which are "repurposed" to compensate for reduced or lost critical ribosomal domains. These include members of the ApiAP2 family which have expanded in apicomplexan and related organisms and were till date only known to function as transcription factors involved in stage conversion.

16:30 – 16:40

Deep mutational resistance profiling for anti-trypanosomal proteasome inhibitors

Simone Altmann, Michele Tinti, Melanie Ridgway, Manu De Rycker, Michael Thomas, Cesar Mendoza Martinez, Jagmohan Saini, Peter Ibrahim, Mike Bodkin and David Horn

Wellcome Centre for Anti-Infectives Research, University of Dundee

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

Poster session 1 (Odds) – 11:10-12:30

1. Dancing out of step: The adaptability of Anopheles stephensi rhythms and their impact on malaria development

Aidan J. O'Donnell & Sarah E. Reece.

Institute of Ecology and Evolution, School of Biological Sciences, University of Edinburgh

Malaria parasites exhibit asexual development that is synchronized with host feeding time and the development of transmission stages are also synchronized with the biting time of the mosquito vector. Disruption of parasites synchrony in the mammalian host results in a reduction of growth and transmission stages, greatly reducing transmission potential to mosquitoes. However, how mosquito rhythms affect parasite development remains uncertain. Moreover, field observations reveal the adaptability of mosquitoes' daily rhythms e.g. adjusting feeding times in response to bed nets and thriving amidst urban environments flooded with artificial light at night. Yet, the potential fitness repercussions for mosquitoes with altered rhythms remain unexplored. Here we disrupted the rhythms of An. stephensi by using photoschedule durations (i.e total hours of day+night) that are longer or shorter than 24 hours and investigate the consequences for mosquito life history traits such as egg lay, survival, nutrition and the timing of flight activity. Second, we explore the impacts of this altered environment for malaria parasite development including the likelihood of infection, the duration of development, and overall parasite density. By unraveling the intricate interplay between biological rhythms, parasite development, and vector behavior, our study offers insights into the complex landscape of infectious disease transmission.

3. FIT plays a role in iron acquisition in Toxoplasma gondii

Dana Aghabi [1], Cecilia Gallego Rubio [1,2], Miguel Cortijo-Martinez [1], Hiram Castillo [3] and Clare R. Harding [1]

1. Wellcome Centre for Integrative Parasitology, Institute of Infection and Immunity, University of Glasgow, Glasgow; 2. University of Oxford; 3. ID21, European Synchrotron Radiation Facility, Grenoble, France.

The obligate intracellular apicomplexan parasite Toxoplasma gondii requires iron as a cofactor for essential metabolic proteins. Due to its importance to almost all life, iron uptake and regulation have been well studied across model eukaryotes. Iron acquisition has been studied in some detail in parasitic kinetoplastids like Leishmania. However, despite its importance, little is known about how Toxoplasma gondii acquires iron. Many organisms make use of specific transporters, named Zinc and Iron Permeases (ZIPs) to take up iron from their environments. T. gondii encodes four ZIP-domain containing proteins, one of which, named here as FIT, is predicted to localize to the plasma membrane and is essential for parasite growth. We endogenously tagged FIT with 3HA tags and show that its localization is dynamic, localizing initially peripherally, before moving basal during parasite replication. Overexpression of FIT provides a protective effect when iron is depleted, but cells are hypersensitive to excess iron, when compared to wildtype parasites. Interestingly, ICP-MS experiments show that FIT overexpression leads to Zn accumulation in the parasite, however no change was seen in parasite associated iron. To determine function, we conditionally depleted of FIT, which led to a severe growth defect in vitro, suggesting it is essential for parasite survival. X-ray fluorescence microscopy (XFM) data show that FIT knockdown leads to reduced parasite iron. Moreover, ICP-MS data show that knockdown of FIT results in a decrease in Zn in the parasites. Finally, we show that knocking down FIT leads to an increased expression of bradyzoite markers including BAG1 and DBL, suggesting that knockdown of FIT may lead to the conversion/differentiation of tachyzoite parasites to the cyst-forming bradyzoite form of the parasite. Interestingly, depletion of iron by chelation also promotes differentiation, suggesting a similar mechanism and a role for iron in triggering differentiation. Together, we show that FIT has a role in iron uptake, and possibly zinc uptake in Toxoplasma gondii, and that this is important for parasite survival in vitro.

5. A role for VSG mRNA in driving allelic competition

Douglas O Escrivani [1], Michele Tinti [1], Jane Wright [1], Sebastian Hutchinson [2], Anna Trenaman [1] Joana R C Faria [3], David Horn [1]

1. Wellcome Centre for Anti-Infectives Research, University of Dundee; 2. Institut Pasteur, Paris; 3. Department of Biology, University of York

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

7. Interrogating genetic determinants of P. falciparum vector transmission

Duangkamon Loesbanluechai [1], Lauriane Sollelis [2,3], Amelia Cox [1], Lizzie Bridget Tchongwe Divala [3], Barbara Stokes [3], Sabyasachi Pradhan [1], Prince Chigozirim Ubiaru [1], Matthias Marti [2,3], Lisa Ranford-Cartwright [1], Virginia Howick [1]

1. School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow; 2. Institute of Parasitology, University of Zurich; 3. School of Infection and Immunity, University of Glasgow.

Plasmodium falciparum parasites are responsible for the most virulent form of human malaria. These parasites are geographically widespread and transmitted through bites of infected Anopheles mosquitos. The abundance and species composition of these susceptible vectors varies geographically, and regional parasite population structure has also been described. This differentiation in both vector community composition and parasite populations could in turn lead to marked parasite-vector adaptations and hence the selection of parasite genotypes that are fit for transmission in distinct local vector communities. Phenotypic evidence for local adaptation of P. falciparum strains has indeed been demonstrated, but the genetic determinants of such interactions are not well elucidated. We combined population genomic data with gene expression studies from the Malaria Cell Atlas to identify a set of P. falciparum candidate loci, which we hypothesize may play a role in adaptation of the parasites to the vector. We tested the role of these naturally occurring candidate polymorphisms in transmissibility to four different vector species from different geographic regions. We found that a subset of the alleles has a vector species-dependent effect on transmission. This information could help elucidate mechanisms of parasite vector co-evolution and inform epidemiological studies and vector control efforts.

9. Quinoxaline-Based Anti-Schistosomal Compounds Have Potent Anti-Plasmodial Activity

Mukul Rawat [1,2], Gilda Padalino [3,4], Edem Adika [1], Tomas Yeo [5,6], Andrea Brancale [7], David A. Fidock [5,6], Karl F. Hoffmann [3], Marcus C. S. Lee [1,2]

1. Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-Infectives Research, University of Dundee; 2. Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom; 3. Department of Life Sciences (DLS), Aberystwyth University, Aberystwyth, United Kingdom; 4. Swansea University Medical School, Swansea, United Kingdom; 5. Department of Microbiology and Immunology, Columbia University Irving Medical Center, New York, New York, United States; 6. Center for Malaria Therapeutics and Antimicrobial Resistance, Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, New York, New York, United States; 7. Department of Organic Chemistry, UCT Prague, Prague, Czech Republic

The human pathogens Plasmodium and Schistosoma are each responsible for over 200 million infections annually, being particularly problematic in low- and middle-income countries. There is a pressing need for new drug targets for these diseases, driven by emergence of drug-resistance in Plasmodium and the overall dearth of drug targets for Schistosoma. Here, we explored the opportunity for pathogen-hopping by evaluating a series of quinoxaline-based anti-schistosomal compounds for activity against P. falciparum. We identified compounds with low nanomolar potency against 3D7 and multidrug-resistant strains. In vitro resistance selections using wildtype and mutator P. falciparum lines revealed a low propensity for resistance. Only one of the series, compound 22, yielded resistance mutations, including point mutations in a non-essential putative hydrolase pfqrp1, as well as copy-number amplification of a phospholipid-translocating ATPase, pfatp2, a potential target. Notably, independently generated CRISPR-edited mutants in pfqrp1 also showed resistance to compound 22 and a related analogue. Moreover, previous lines with pfatp2 copy-number variations were similarly less susceptible to challenge with the new compounds. Finally, we examined whether the predicted hydrolase activity of PfQRP1 underlies its mechanism of resistance, showing that both mutation of the putative catalytic triad and a more severe loss of function mutation elicited resistance. Collectively, we describe a compound series with potent activity against two important pathogens and their potential target in P. falciparum.

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

Melanie C. Ridgway, Michele Tinti & David Horn

The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee.

Acoziborole is a safe, single dose, oral therapy, suitable for treatment of both early and late-stage human African trypanosomiasis. This drug is currently under development for paediatric application, while other oxaboroles show efficacy against trypanosomatids, Apicomplexans and fungi. Acoziborole and several other oxaboroles targets cleavage and polyadenylation specificity factor 3 (CPSF3; see PMID: 30185555). We developed oligo targeting for rapid and precision editing in otherwise wild type trypanosomatids (PMID:35524555) and subsequently found that the method could be used to introduce multiple edits in one step. We've now used the approach for saturation mutagenesis around the drug-binding, and CPSF3 catalytic pocket, and for multi-editing. Among >1000 edits affecting residues within 5 Å of the acoziborole binding site, only the pair of Asn232His edits conferred moderate resistance to acoziborole or the related oxaborole DNDI-6148. By targeting multiple codons simultaneously, however, we found additive or synergistic acoziborole resistance-conferring edits. Together with a Asn232His edit, Tyr383Phe, and Asn448 edits increased resistance relative to the Asn232His edit alone. These multi-edited sites are homologous to AN3661 resistance-conferring mutations described in Plasmodium falciparum and Toxoplasma gondii. This study highlights the versatility of oligo targeting and provides new insights into CPSF3-associated acoziborole resistance.

13. How do biological rhythms impact parasite infection and transmission in wild wood mice?

Aniela M Dexter, Amy B Pedersen, Sarah E Reece

Institute of Evolution Ecology, School of Biological Sciences, University of Edinburgh.

Circadian clocks enable organisms to respond to and prepare for cyclical changes in the biotic and abiotic environment. For example, immune functions are often upregulated prior to and during an organism's active phase—when hosts are more likely to encounter parasites. Parasites can also exhibit rhythmic behaviors that influence their within host survival and between host transmission, although to what extent these rhythms stem from hosts or the parasites themselves is poorly understood. Biological rhythms are plastic and affected by infection, food availability fluctuations, and seasonal temperature changes. However, how such shifts in rhythms impact parasite infection and transmission is underexplored, especially in natural populations. Wood mice (Apodemus sylvaticus) present a particularly tractable system to investigate the roles of host activity and feeding/fasting rhythms in infection because their interactions with parasites in the wild are well characterized and because methods for investigating rhythms are well developed in mice. I present my plans to combine field and laboratory experiments to probe how perturbation of rhythms influences the spread and severity of parasitic infections.

15. Chemical pulldown of anti-malarial TCMDC-135051 identifies unexpected target in Cryptosporidium

Grant M J Hall, Aisha Syed, Victor Corpas-Lopez, Gourav Dey, Robert Smith, Stephen Patterson, Susan Wyllie, Mattie Christine Pawlowic

Wellcome Centre for Anti-Infectives Research, University of Dundee

Cryptosporidium is one of the leading pathogens associated with diarrheal mortality and morbidity in children, with no effective drug available. Several drug discovery efforts have produced new potential therapeutics, however most were initiated from phenotypic screens and these programmes lack information about the compound's target. To ensure clinical success, this gap in knowledge must be addressed. Chemical pulldowns have been applied in other eukaryotic parasites to identify a compound's molecular target(s). This is an unbiased technique to identify proteins that bind and physically interact with a compound. Proteins are identified by quantitative mass spectrometry. We incubate C. parvum lysate with compounds that are immobilised onto beads; by competing with free compound, we can determine which proteins specifically interact with the compound of interest. To optimise this method for C. parvum we used TCMDC-135051, a compound originating from the TCAMS set and active in Plasmodium. We were able to successfully pulldown proteins that interact with TCDMDC-135051. However, we identified the target to be CpCRK2, not the homolog of the target in Plasmodium, PfCLK3. This provides critical information about the potential of this molecule as an anti-cryptosporidial and provides a new technology to identify the target of other phenotypic hits.

17. The relationship between expelled eggs, morbidity and age: Implications for current S. mansoni elimination policies

Rivka M. Lim [1*], Ruhi Lahoti [1], Moses Arinaitwe [2], Victor Anguajibi [3], Andrina Nankasi [2], Fred Besigye [2], Alon AtuhAire [2], Amy B. Pedersen [1], Joanne P. Webster [4•], Poppy H.L. Lamberton [5•*]

1. Institute of Evolution and Ecology, School of Biological Sciences, Ashworth Laboratories, University of Edinburgh; 2. Vector Borne and Neglected Tropical Diseases Control Division, Ministry of Health, Kampala, Uganda; 3. China-Uganda Friendship Hospital, Kampala, Uganda; 4. Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London; 5. School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow.

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•Joint authorship

Direct assessment of morbidity is rarely included during monitoring and evaluation of Schistosoma mansoni control or elimination programmes. Instead, the number of eggs per gram (EPG) of faeces is used as a proxy for morbidity. The World Health Organization has targets for schistosomiasis elimination as a public health problem (EPHP) by 2030, defined as <1% heavy infections (classed as ≥400 EPG). However, recent findings challenge the assumed link between current infection intensity and morbidity.

Prevalence and intensities of S. mansoni were diagnosed by Kato Katz and point-of-care circulating cathodic antigen tests in 287 individuals, 3-74 years old, from Bugoto, Uganda. Ultrasound examinations following the Niamey protocol were used to characterise periportal fibrosis (PPF), portal vein dilation (PVD) and left parasternal line (PSL) enlargement. Additional morbidity markers included anaemia and self-reported symptoms. Logistic regression models were used to elucidate potential predictors of morbidity.

PPF, PVD, PSL and anaemia prevalence were 9%, 34%, 33% and 13% respectively. School-aged children (SAC) had the highest infection intensity, but pre-school-aged children (PSAC) were significantly more likely to have PVD, PSL and anaemia than all other age groups. Current S. mansoni infection was only predictive of the self-reported symptom of blood in stool. As infection intensity increased, so did the likelihood of anaemia and fibrosis, but this was only significant at levels much higher that the 400 EPG threshold. Current malaria was associated with PVD and anaemia.

Our findings add to growing evidence against the continued use of ≥400 EPG as a proxy for schistosomiasis morbidity, urging a revaluation of targets. Moreover, the age-related distribution of morbidities observed, with a notable burden in PSAC, highlights a critical need to elucidate the impact of less-specific morbidities on host health and its interplay with current and past infections with both S. mansoni and other parasites.

19. Targeting epigenetic regulators in Plasmodium

Jed Hawes, Marcus Lee

Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee

Malaria remains a major global health challenge, causing over 200 million infections annually. The growing resistance to existing antimalarial drugs emphasizes the urgent need for novel therapies with new modes of action. Plasmodium, the causative parasite, has a complex life cycle, with gene expression tightly regulated by various epigenetic mechanisms. Targeting epigenetic regulators could provide potential therapeutic strategies. My research focuses on identifying essential epigenetic regulators in Plasmodium that may serve as effective drug targets. Additionally, I aim to screen epigenetic inhibitors, known to work in other eukaryotic systems, to explore their potential against Plasmodium and unravel their mechanisms of action. Specifically, I am studying an understudied and parasite-specific bromodomain protein, BDP6. Bromodomain-containing proteins (BDPs), which bind to acetylated lysine residues on histones and regulate transcription, have already been identified as promising drug targets in other diseases. Conditional knockout of PfBDP6 reveals its essentiality during the blood stage, indicating it as a promising drug target. We are currently purifying recombinant BDP6 to determine which histone residues it binds. In parallel, I am investigating two other epigenetic proteins, PfMyst and PfSET3, using knockout approaches to assess their drug target potential. Furthermore, I have identified several potential epigenetic inhibitors for Plasmodium and am performing in vitro drug resistance selection to explore their mechanisms of action. This research aims to uncover new therapeutic candidates and shed light on novel druggable pathways in malaria treatment.

21. Permeability and morphological dynamics during Plasmodium falciparum gametocyte maturation.

Jahiro Gómez [1], Priscilla Ngotho [2,3], Lorena Coronado [1] and Matthias Marti [2,3]

1. Institute for Scientific Research and High Technology Services (INDICASAT-AIP), Panama City, Panama; 2. Wellcome Centre for Integrative Parasitology, University of Glasgow, Glasgow, United Kingdom; 3. Institute of Parasitology, Vetsuisse Faculty, University of Zurich.

During Plasmodium falciparum sexual development, the gametocyte (the sexual and transmissible parasite stage) extensively modifies its erythrocyte host cell leading to morphological and biophysical alterations. These reversible changes take place during gametocyte maturation in the haematopoietic niche of the bone marrow, before the mature gametocyte re-enters the bloodstream for transmission to the mosquito. For example, tubulin, a key component of the parasite's cytoskeletal network, undergoes dynamic remodeling throughout gametocyte maturation. In this study, we use immunofluorescence assays (IFA) to track tubulin dynamics and localisation across the developmental stages of gametocytes. For this, we have used commercially available tubulin tracker to label polymerized tubulin filaments. Our findings show that tubulin labeling intensifies during gametocyte maturation, and this intensity is similar across different P. falciparum strains. Additionally, we have used staining against the host cytoskeletal marker a-spectrin to show that host membrane integrity is maintained despite major remodeling during gametocyte development. Understanding these well-regulated structural changes will provide insight into the mechanisms that enable Plasmodium falciparum to thrive within its host and inform potential transmission blocking strategies.

23. Iron deprivation flips a metabolic switch towards anaerobic metabolism in *Toxoplasma gondii*

Jack Hanna and Clare R. Harding

Centre for Parasitology, School of Infection and Immunity, University of Glasgow

Iron is essential for almost all life due to its role as a cofactor in numerous biological processes. Excess iron is however toxic, meaning regulation of cellular iron abundance is critical. For *Toxoplasma gondii,* iron must be obtained from their host cells. Unprecedented host cell promiscuity means it must contend with diverse and dynamic levels of accessible iron. To investigate how Toxoplasma regulate and respond to changing iron availability, we used quantitative proteomics to determine the impact of iron deprivation on the *Toxoplasma* proteome. We analysed changes to 4742 proteins from *Toxoplasma* tachyzoites and found that 1373 proteins had significant changes in their abundance. Of these significant changes, 835 proteins were downregulated, suggesting a general decrease in protein abundance in response to iron deprivation. Iron deprived parasites exhibited downregulation of translation machinery including the key ribosome recycling factor ABCE1 and an accompanying reduction to translation measured by puromycin incorporation. While exhibiting translational repression, iron deprived parasites alter their energy metabolism. Subsequent metabolic analysis determined that iron deprived *Toxoplasma* exhibit a reduction in mitochondrial oxygen consumption while maintaining glycolytic output. These data highlight the importance of iron to diverse processes and indicate that while downregulating protein synthesis, Toxoplasma also rewire their metabolism in response to iron deprivation.

Poster session 2 (Evens) – 14:30-15:50

2. Developing a Cre & DiCre conditional gene expression system to investigate essential C. parvum genes

Kai Lynn Wong [1], Simona Seizova [1,2], Lee Robinson [1], Bethany Dow [1], Emma Carrington [1,3], Mattie Christine Pawlowic [1]

1. Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee; 2. Walter and Eliza Hall Institute of Medical Research, Infectious Diseases and Immune Defence, Melbourne, Australia; 3. University of St Andrews

The gastrointestinal parasite Cryptosporidium is a waterborne pathogen and is one of the leading causes of mortality caused by diarrhoeal disease in young children and immunocompromised individuals. Currently, there are no effective treatments or vaccines available. In this project, we develop the next generation of the Cre and DiCre conditional gene expression system in Cryptosporidium parvum. We use CRISPR to introduce loxP sites flanking regions of the genome of interest. Then Cre/DiCre is introduced at a second locus, and when expressed excises the region between the loxP sites. We utilise our new selection marker to generate both floxed and Cre/DiCre strains and introduce both mutations by genetic cross. Our new generation of strains express both NanoLuciferase (NLuc) and Firefly Luciferase (Fluc). These reporters allow us to track oocyst shedding (NLuc) and the parasite infection of the gastrointestinal track (Fluc and in vivo imaging) to determine impact of gene loss on transmission specifically. CDPK1 was identified to be an essential gene in C. parvum. We use CDPK1 as a proof of concept on the development of Cre/DiCre strains. We aim to develop a regulatable gene expression system to validate potential Cryptosporidium drug targets and explore parasite transmission biology.

4. Unravelling the Molecular Mechanisms of Toxoplasma gondii Complex II

Kiera Douglas, Mariana F. Silva, Andrew Maclean, Amit Meir, David Bhella, Lilach Sheiner,

School of Infection and Immunity, University of Glasgow

The four mitochondrial respiratory complexes (II, III, IV and V) of Toxoplasma gondii are essential for energy metabolism and survival. Of particular interest, is T. gondii complex II (succinate dehydrogenase, TgCII) due to it being one of the entry points to the electron transport chain (with the absence of complex I from T. gondii) and the only link to the TCA cycle. Our previous studies have proposed seven putative new subunits in TgCII for a total of nine, more than the usual four subunits found in mammals and yeast [1]. The contribution of these subunits to TgCII have been experimentally validated through microscopy, cell-biology, and biochemical methods [2], raising the question of what the functional implications of the new complex composition are. Importantly, two of the canonical four subunits, that make critical contribution to the catalytic sites, don't seem to have clear homologs in the T. gondii complex. Furthermore, structural predictions do not produce a structure that includes the required active sites for TgCII known activity. Sequence alignments provided some progress by revealing that one of the subunits, S10, contains a highly conserved DY motif involved in activity, and this was validated via genetics. To complete the picture and elucidate the molecular mechanisms of TgCII function, we plan to solve the structure using single particle analysis via cryoEM. Here I show my progress in optimising purification of TgCII, and of the data analysis pipelines via single particle analysis.

8. Cryptosporidium parvum infection is much more widespread throughout the calf GI than previously assumed.

Goddard, P. [1], Tzelos, T. [2], Bartley, P.M. [2], Colon, B.L. [1], Robinson, L., [1], Hall, G.M.J. [1], Smith, D*. [2], Katzer, F*. [2], Pawlowic, M.C*. [1]

1. Wellcome Centre for Anti-Infectives Research, Division of Biochemistry and Drug Discovery (BCDD), School of Life Sciences, University of Dundee; 2. Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh.

Zoonotic parasite Cryptosporidium parvum infects both humans and ruminants (particularly cattle), causing the diarrhoeal disease cryptosporidiosis. Infection can cause severe clinical symptoms, including profuse watery diarrhoea, growth stunting, and even death. Limited treatment is available, yet ineffective; this is true for both humans and cattle. Taking a One Health perspective is key as cattle are a documented source of human disease and environmental contamination, implicated in the recent outbreak in Devon. Despite the risks C. parvum poses, our current understanding of infection in calves is lacking, with foundational knowledge such as the parasite's location in the gut unknown, and host-pathogen interaction poorly understood.

To address these critical knowledge gaps, we conducted a first-of-its-kind study infecting neonatal calves with transgenic reporter parasites. This allowed us to measure parasite burden throughout the gut and identify the location(s) of infection. The prevailing hypothesis was that, in cows, infection localises specifically to the ileo-caecal junction, yet this lacked robust experimental evidence. Our findings challenge this notion; we observed that infection is much more widespread throughout the length of the GI. Now that we appreciate the breadth of infection, we are interrogating host-pathogen interaction via dual host and parasite transcriptomics samples, collected from our study.

10. Functional control via redox regulation and potential inhibition of the divergent Toxoplasma gondii succinate dehydrogenase (mETC complex II)

Mariana F. Silva [1], Wasim Hussain [1,2], Kiera Douglas [1], Andrew E. Maclean [1], Lilach Sheiner [1]

1. School of Infection and Immunity, University of Glasgow; 2. Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital

The mitochondrial electron transport chain (mETC) of Toxoplasma and other apicomplexans is an important source of new targets for drug discovery for apicomplexan diseases. Our recent work identified numerous new components of the T. gondii mETC complex II/ succinate dehydrogenase (SDH) (PMID: 33651838). We validated this unusual composition through localisation, genetic and biochemical studies of five new subunits where we demonstrated their importance for parasite growth, SDH complex integrity and its enzymatic activity (PMID: 38079448). The question is now, what is the role of the new components in the function and regulation of this essential and divergent parasite complex?

In other systems SDH activity is controlled by Thioredoxins (Trxs)(PMID: 33072147; PMID: 25646482). Trxs are enzymes that controls the activity and localisation of proteins in response to redox changes in cellular compartments through disulfide exchange. Here we identify and characterise a mitochondrial thioredoxin (Mtrx1) that may mediate SDH activity. We verified its mitochondrial localization, thioredoxin activity, through site-directed cysteine mutagenesis, and mapped its interactions via disulfide-trappings. In line with a role in mETC regulation Mtrx1 depletion results in reduced oxygen consumption.

Overall, our work reveals the mechanism of function of an apicomplexan SDH and discovered the first apicomplexan Mtrx involved in mETC regulation.

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

Markéta Novotná, Michele Tinti, David Horn

Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee.

It remains unclear how transcription, DNA replication and DNA repair rely upon chromatin-based controls in trypanosomatids. Terminal histone tails, and tail modifications, play key roles in these processes in other eukaryotes. However, trypanosomatid histones are highly divergent relative to the usual model eukaryotes. Notably, interpretation of 'writer', 'reader' and 'eraser'-defective phenotypes is complicated by potential perturbation of non-histone substrates. Genetic manipulation and subsequent study of histone functions have also proven challenging because core histone genes are present as many identical copies of each gene.

We used an inducible CRISPR/Cas9 system in Trypanosoma brucei to delete all native copies of the histone H4 genes, complementing the defect with a single, recoded and highly expressed ectopic copy. Further templated editing was then used for site saturation mutagenesis of lysine residues (K4, K10 and K14) in the N-terminal tail of the ectopic H4 gene. Multiplex amplicon-seq profiling was used to monitor relative fitness, revealing those tolerated H4-K4 or H4-K14 mutations; H4-K10 mutations were not tolerated. A panel of strains exclusively expressing novel histone H4 mutants, including arginine (R; non-acetylated mimic) or glutamine (Q; constitutively acetylated mimic) substitutions, was phenotypically profiled; using proteomic, transcriptomic, microscopy, growth, protein blotting, and flow cytometry. We observed a modest but specific defect in Variant Surface Glycoprotein gene silencing in H4-K4Q mutants – the first direct evidence that histone tails and their modification impact this process.

14. Exploring mutator phenotypes to facilitate the understanding of Plasmodium falciparum antimalarial drug resistance

Edem Adika & Marcus Lee

Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee

New antimalarial drugs are required to counter the current threat of antimalarial drug resistance. In vitro resistance generation is invaluable in uncovering the mechanism of action or resistance of antimalarial compounds. Furthermore, the frequency of resistance generation, in particular the minimum inoculum of parasites for resistance (MIR), is a valuable approach to understand potential resistance risks during drug development. However, these approaches require large parasite numbers, and frequently the inability to generate resistance for some antimalarial compounds in vitro. A recent breakthrough from our laboratory has generated a mutator parasite with increased mutation rate and higher genetic diversity. This necessitates validation and standardization of this line for quantitative in vitro resistance generation. The aims of this research are to miniaturize the MIR assay and enhance the P. falciparum mutator phenotype.

In vitro resistance generation to DSM265, KAE609, GNF179 and atovaquone with Dd2 wildtype and DNAPolδ mutator parasites returned recrudescent parasites at different inoculum sizes, with the mutator parasite yielding parasites at lower inoculum sizes for KAE609 and GNF179. Recrudescent parasites showed varied fold-shifts in IC50s from the parental lines indicating multiple distinct mutations.

Thus, preliminary results show that the mutator parasite can generate resistance to a range of antimalarial compounds at a lower MIR compared to the wildtype Dd2 strain. Further improvements to the mutator phenotype should enable generation of resistance to more antimalarial compounds. To date, CRISPR/Cas9 editing has successfully generated a conditional knockdown mutant of pfdnapolε subunit B tagged with a glmS ribozyme, which will be evaluated using the MIR assay. This approach should also allow for generation of resistance on a higher throughput scale.

16. Do rhythmic interactions between mosquitoes and their microbiota influence malaria transmission?

Naomi Riithi [1], Jason P. Mooney [2] & Sarah E. Reece [1]

1. Institute of Evolution Ecology, School of Biological Sciences, University of Edinburgh; 2. Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh.

Mosquitoes are the most important vectors of infectious disease, transmitting not only malaria parasites (Plasmodium spp.) but also a variety of lethal pathogens such as Dengue Fever, West Nile virus, Yellow Fever, Zika virus and Chikungunya virus, among others. Mosquito symbionts (microbes) are increasingly being recognised as crucial and influential players in vectorial capacity – a measure of transmission potential. Mosquito gut-microbiota modulate the vector immune system and produce antiparasitic proteins to block Plasmodium transmission. Therefore, the microbial community in the mosquito conveys critical roles in vector development and influences the survival and fecundity of adults. Mosquitoes exhibit a daily rhythm when they forage for blood or sugar, which imposes a rhythm on the diversity and abundance of the microbial community. This, in turn, may generate rhythmicity in the impacts of mosquito immune responses and microbial-produced antiparasitic proteins on Plasmodium transmission. Nevertheless, the potential for microbe-related rhythms to influence parasite development and affect mosquito vectorial capacity remains unknown. Here, I will discuss the experimental approaches needed to address this gap. Since mosquito-biting times of day are changing, understanding how rhythms impact on host-parasite-vector interactions is critical. Demonstrating the impact of microbial rhythms in transmission could inform malaria control and drive novel interventions.

18. Antimicrobial resistance and Contaminated soils

Sarah I. Bassey, Iain McLellan, Kiri Rodgers, Andrew Hursthouse and Fiona Henriquez-Mui.

University of the West of Scotland

Antimicrobial resistance (AMR) is the inability of micro-organisms to respond to antimicrobial treatments.

Potentially toxic elements (PTEs) are key drivers of AMR in the soil environment, as they can contribute to the establishment of resistant genes in soils. Metals such as Cr, Cu, Fe, Ni, Mn, and Zn in high concentrations acts as antimicrobials.

The persistence of PTEs in soil environments enables prolonged selection pressure, outlasting that of pharmaceutical and clinical compounds. Moreover, pollutants from industrial and urban sources significantly amplify the scale of this selection process, making it more extensive than any other contributing factor.

Soil properties such as pH and organic matter affects the mobility and availability of the PTEs within the soil column, which in turn impacts on PTE toxicity to the microbiome. Therefore, soil / sediment samples collected from contaminated sites would be analysed for physicochemical properties and microbiological characteristics. The goal is to better understand the relationship between PTEs and microorganisms, as well as the selection pressure exerted by PTEs in driving resistance.

This research aims to address existing knowledge gaps in this area, and explore opportunities to integrate AMR considerations into strategies for remediating pollution legacies and controlling waste entry into the soil environment.

20. Molecular Characterization of Opisthorchis viverrini TGF-beta Homologue and Role in Host-Parasite Interactions

Nuttanan Hongsrichan [1,2], Claire Ciancia [1], Kyle Cunningham [1], Anna Sanders [1], Rick M. Maizels [1]

1. Wellcome Centre for Integrative Parasitology, School of Infection and Immunity, University of Glasgow. 2. Department of Parasitology, Faculty of Medicine, Khonkaen University, Thailand.

Opisthorchis viverrini, a human liver fluke, is a causative agent of opisthorchiasis and a significant risk factor for cholangiocarcinoma. Parasites employ various strategies, including secretion of proteins that engage host molecules, to evade the host immune response, making these molecules promising targets for vaccine and drug development. The transforming growth factor-β (TGF-β) signaling pathway plays a pivotal role in numerous cellular processes, including growth, differentiation, apoptosis, motility, invasion, and immune response regulation. While homologues of TGF-β have been identified in helminth parasites, the characterization of O. viverrini-derived member of the TGF-β superfamily has been limited.Our study aimed to construct a recombinant TGF-β homologue molecule of O. viverrini (OV-TGH) and elucidate its role in host-parasite interactions and immune modulation. The sequence encoding O. viverrini TGF-β was cloned into the pSecTag2A expression vector and expressed using the Expi293 mammalian cell expression system. The recombinant OV-TGH molecule's activity was assessed through the MFB-11 assay and its involvement in the TGF-β signaling pathway was investigated. This study contributes to a deeper understanding of the role played by the TGF-β homologue molecule of O. viverrini in host survival and immunopathogenesis, shedding light on potential therapeutic targets for opisthorchiasis and associated complications.

22. Characterising the Oocyst Wall Proteins at the Cryptosporidium suture

Sarah Stevens [1], Ross Bacchetti [1,3], Leandro Lemgruber [2], Lee Robinson [1], Mattie Christine Pawlowic [1]

1. Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee; 2. Cellular Analysis Facility, MVLS-Shared Research Facilities, College of Medical, Veterinary & Life Sciences, University of Glasgow; 3. Scottish Microbiology Reference Laboratories, Glasgow Royal Infirmary.

Cryptosporidium is a waterborne, protozoan parasite that is a leading cause of diarrhoeal disease that lacks effective treatments and vaccines. Parasites are often transmitted in water inside an eggshell-like structure called an oocyst. The oocyst wall provides protection against disinfectants and common water treatments, including chlorination. Once ingested by a host, four parasites emerge from a zipper-like opening in the oocyst wall called the suture. How they build this suture and then "unzip" is unknown.

We lack experimentally validated molecular markers of the oocyst wall. Additionally, there are no markers for the suture. Using CRISPR we made transgenic fluorescent reporter strains and confirmed that all members of the predicted Cryptosporidium Oocyst Wall Protein (COWP) family localise to the oocyst wall in Cryptosporidium parvum (Bacchetti et al biorxiv, 2024).

Among this family we identified the first markers of the oocyst suture. We used Imaris (3D image analysis software) to analyse fluorescence localisation. The signal is ~5.5 microns long and spans approximately one-third of the oocyst circumference. Suture shape and length were consistent across all reporter strains and in agreement with previous measurements of the suture from electron microscopy data. We will report our further image-based characterisation of these new suture proteins.

24. ATR, a DNA damage kinase, modulates DNA replication timing by ensure the progress of the process over potential genomic replicative stress regions in Leishmania major.

G Almeida da Silva [1,2], J D Damasceno [1], J A Black [2], R McCulloch [1], L R Tosi [2]

1. University of Glasgow Centre for Parasitology, School of Infection and Immunity, University of Glasgow; 2. Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

All cells possess mechanisms to maintain and replicate their genomes, whose integrity and transmission are constantly challenged by DNA damage and replication impediments. In eukaryotes, the protein kinase Ataxia-Telangiectasia and Rad3-related (ATR), a member of the phosphatidylinositol 3-kinase-like family, ensures genome maintenance and stability, and is considered as a master regulator of the eukaryotic response to DNA injuries. Here we aimed to investigate the conservation and functional relevance of the ATR homolog in the DNA metabolism of Leishmania major, a protozoan parasite with a remarkably plastic genome. CRISPR/cas9 genome editing was used to generate a Myc-tagged ATR cell line (mycATR), and a Myc-tagged C-terminal knockout of ATR (mycATRΔC-/-). We show that ATR nuclear localisation depends upon the C-terminus and its mutation results in accumulation of single-stranded DNA, impaired cell cycle control, increased levels of DNA damage, and delays DNA replication re-start after replication stress. In addition, we show that ATR is vital for the maintenance of L. major's DNA replication program, where small chromosomes replicate earlier than larger ones; specifically, ATR mutation results in increase of replication signal around regions with overlapping of Switch Strand Regions (SSRs) and repetitive sequences after replicative stress. Finally, we show that such changes in the DNA replication program leads to chromosome instability and affects genome variability. In summary, our work shows that ATR is involved in the crucial balancing of accurate DNA replication around potential replicative stress regions, and such activity is central for the integrity and variability of Leishmania genome.

Attendee list

Mohamed	Abdelrahim	University of Edinburgh
Temitayo	Ademolue	University of Edinburgh
Praise	Adeyemo	University of Glasgow
Edem	Adika	University of Dundee
Dana	Aghabi	University of Glasgow
Simone	Altmann	University of Dundee
Lilly	Atkins	University of Glasgow
Ross	Bacchetti	NHS Scotland
Christa	Baker	University of Dundee
Sarah	Bassey	University of the West of Scotland
Tera	Birchall	University of Dundee
Peter	Black	University of St Andrews
Josefina	Bonomi	Institut Pasteur de Montevideo
Nonlawat	Boonyalai	University of Dundee
Gustavo	Bravo Ruiz	University of Dundee
Collette	Britton	University of Glasgow
Laurine	Brouck	University of Dundee
Flora	Caldwell	University of Edinburgh
Paul	Campbell	University of Glasgow
Marta	Campillo	University of Glasgow
Emma	Carrington	University of St Andrews
Saniya	Crouch	Moredun Institute
Li An	Crowley	University of Glasgow
Kyle	Cunningham	University Of Glasgow
Gabriel	Da Silva	University of Glasgow
Cristina	del Rio Cubilledo	University of Edinburgh
Aniela	Dexter	University of Edinburgh
Samuele	Di Carmine	University of Dundee
Kiera	Douglas	University of Glasgow
Jack	Duggan	University of Dundee
Neil	Duncan	University of Edinburgh
Rebecca	Edgar	University of Dundee
Douglas	Escrivani De Oliveira	University of Dundee
Rosie	Fellows	University of Glasgow
Ximena Danay	Fernandez Hual	University of Edinburgh
Olivia	Fleming	University of Edinburgh
Oscar	Forestier	University of Edinburgh
Matt	Gibbins	University of Glasgow
Beric	Gilbert	University of the West of Scotland
Grace	Gill	University of Glasgow
Peyton	Goddard	University of Dundee
Jahiro	Gómez	University of Glasgow
Grant	Hall	University of Dundee
Jack	Hanna	University of Glasgow
Clare	Harding	University of Glasgow
Elena	Hartmann	University of Edinburgh

Kedir	Hassen	Eduardo Mondlane University
Jed	Hawes	University of Dundee
Anna	Heawood	University of Glasgow
Ben	Holdsworth	University of Edinburgh
Nuttanan	Hongsrichan	University of Glasgow
Virginia	Howick	University of Glasgow
Rhoslyn	Howroyd	University of Edinburgh
Benedict	Karani	University of Glasgow
Brenda	Karumbo	University of Glasgow
Trisha	Kerai	University of Edinburgh
Martha	Kivecu	University of Edinburgh
Marija	Krasilnikova	University of Glasgow
Marcus	Lee	University of Dundee
Rivka	Lim	University of Edinburgh
Duangkamon	Loesbanluechai	University of Glasgow
Andrew	Maclean	University of Glasgow
Peter	McBride	University of Dundee
Andrew	McCluskey	University of Glasgow
Jennifer	McIntyre	University of Glasgow
Andreia	Moniz E Castro	University of Glasgow
Mackenzie	Moore	University of Edinburgh
Lucia	Mrvova	University of Glasgow
Jane	Munday	University of Glasgow
Marketa	Novotna	University of Dundee
Aidan	O'Donnell	University of Edinburgh
Guy	Oldrieve	University of Edinburgh
Hoyam	Osman	University of Edinburgh
Jana	Ovciarikova	University of Glasgow
Lucas	Pagura	University of Glasgow
Luciana	Paradela	University of Dundee
Mattie	Pawlowic	University of Dundee
Chloe	Pelletier	University of Dundee
Lorraine	Pfavayi	University of Edinburgh
Nisha	Philip	University of Edinburgh
Joanne	Power	University of Glasgow
Bethan	Preece	University of Glasgow
Tom	Purnell	University of Glasgow
Abhinay	Ramaprasad	University of Glasgow
Marcos	Ramos	Independent
Mukul	Rawat	University of Dundee
Sarah	Reece	University of Edinburgh
Josh	Richards	University of Dundee
Melanie	Ridgway	University of Dundee
Naomi	Riithi	University of Edinburgh
Emily	Robertshaw-McFarlane	University of Edinburgh
Kiri	Rodgers	University of the West of Scotland
Anna	Sanders	University of Glasgow
Petra	Schneider	University of Edinburgh

Lilach	Sheiner	University of Glasgow
Shikha	Shikha	University of Glasgow
Mariana	Silva	University of Glasgow
Megan	Sloan	University of Glasgow
Amber	Smith	University of Dundee
Pieter	Steketee	University of Edinburgh
Sarah	Stevens	University of Dundee
Aisha	Syed	University of Dundee
Camila	T B Correia	University of Glasgow
Maisha	Tanzim	University of Glasgow
Kenneth	Tay Bing Xiang	University of Dundee
Frank	Venter	University of Edinburgh
Kiran	Wadhawan	University of Edinburgh
Andy	Waters	University of Glasgow
Ruby	White	University of Glasgow
Jamie	Whitelaw	University of the West of Scotland
Charlotte	Willoughby	University of Edinburgh
Kai Lynn	Wong	University of Dundee
Stuart	Woods	University of the West of Scotland