Mechanisms of selective translation stimulation and suppression by the multiple eIF4E isoforms of *Trypanosoma brucei*

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Trypanosoma brucei has six isoforms of the cap-binding translation initiation factor eIF4E, and five eiF4Gs (PMID: 29077018), which potentially allows for differential mRNA target selection in order to fine-tune translation. EIF4E3 and EIF4E4 appear to be general initiation factors and EIF4E2 is dispensable; we are investigating EIF4E1, EIF4E5, and EIF4E6.

EIF4E1 interacts with 4EIP (4E-interacting protein) and not with any EIF4G; they functionally resemble mammalian 4E-HP and GIGYF2. 4EIP suppresses translation and provokes mRNA degradation. 4E-IP and EIF4E1 are dispensable in slender "bloodstream forms", which multiply in mammals, but 4EIP is required for translation suppression in the growth-arrested "stumpy" bloodstream form (PMID: 30124912). Meanwhile EIF4E1, but not 4EIP, is required for survival of "procyclic" forms, which grow in Tsetse. New results suggest that the EIF4E1-4EIP complex recruits the CAF1-NOT deadenylation complex and a cystosolic terminal uridylyltransferase 3 (TUT3). Tethered EIF4E1 is suppressive only when 4EIP is present. We are investigating whether it can, without an eIF4G, activate translation in procyclic forms, and how it and 4EIP select target mRNAs.

EIF4E3, 4, 5 and 6 all stimulate expression when tethered. They interact with different EIF4G homologues, and each is essential in at least one life-cycle stage, indicating that each has a distinct role. EIF4E3 and EIF4E4 are thought to be general translation factors. We have found that EIF4E6 interacts specifically, not only with EIF4G5, but also with a previously characterised stimulatory complex containing MKT1, PBP1 and LSM12. The MKT complex is recruited to mRNAs via sequence-specific RNA-binding proteins (PMID: 24470144), offering a novel mechanism for specific translation activation by the EIF4E6-EIF4G5 complex. Identification of mRNAs that are selected by EIF4E6 is in progress, and we are also interested in the function of EIF4E5.

TbMYND and RNA-Binding Protein 6 (RBP6) as master regulators of *Trypanosoma brucei* differentiation and migration in the tsetse

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The colonisation and migration of *Trypanosoma brucei* through the tsetse is accompanied by a series of developmental changes that are the result of a tightly-regulated programmed gene expression. After the 'stumpy' bloodstream form differentiates into the procyclic stage in the midgut lumen, trypanosomes colonise the midgut ectoperitrophic space via the proventriculus (PV). Later in the PV, the parasite further differentiates into the short epimastigote form, which then establishes a salivary gland infection that leads to the formation of transmissible metacyclic trypomastigotes.

To identify *T. brucei* genes involved with life cycle progression in the tsetse, we used RNA-seq to compare the expression profiles of proventricular trypanosomes from a fly-transmissible strain with that of a strain unable to infect salivary glands. We found >700 up-regulated transcripts in the fly-transmissible strain. The top hit was identified as a conserved hypothetical protein across kinetoplastid organisms, which contain a predicted MYND zinc finger domain (Myeloid, Nervy and DEAF-1) in the C-terminus. Other top hits corresponded to several folate transporters, glutamate dehydrogenase, and RNA-binding protein 6 (RBP6), which is known to trigger differentiation of procyclics into metacyclics *in vitro*. Only the overpression of TbMYND restored infectivity of salivary glands in the impaired strain. While all *Trypanosoma* and *Leishmania* sp. TbMYND homologues conserve the MYND domain, only the one from the primitive *Bodo saltans* has an additional canonical RNA-binding domain in the N-terminus, suggesting that TbMYND may act as an effector accessory protein. In fact, pull-down assays showed that TbMYND interacts with a range of hypothetical proteins, most of them with identifiable zinc finger domains, including MYND-type. Interestingly, overexpression of TbMYND in procyclic cells led to the formation of epimastigotes (but not metacyclics) *in vitro* probably via a secreted factor that is smaller than 3 kDa. Furthermore, *TbMYND* CRISPR KO cells infect poorly the tsetse midgut and are completely unable to colonise the PV.

Given that TbMYND is primarily expressed in PV forms and RBP6 in both the PV and salivary gland forms, we hypothesize that the sequential expression of these proteins coordinates the developmental progression of trypanosomes in tsetse.

Supported by the Wellcome Trust and ITN-FP7 GlycoPar.

The EuPathDB.org Family of Databases for Host-Pathogen Research

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The Eukaryotic Pathogen Database Resources (EuPathDB, http://eupathdb.org) are a family of 12 taxon-specific, free, online genome and other Omics data mining resources that support almost 200 organisms within the Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi and several host species. These resources facilitate the discovery of meaningful biological relationships or testing of hypotheses from large volumes of integrated pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. The graphic interface allows users to take full advantage of the data without the need for computational training. Data types range from genome sequence and annotation to transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using standard bioinformatics workflows and in-house analyses generate data including domain predictions and orthology profiles across all genomes which permit inferences from data-rich organisms to organisms with limited or missing data. EuPathDB offers several perspectives for data mining – record pages compile all data for genes, pathways, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system that queries pre-analyzed data and returns genes or features with shared biological characteristics; a private Galaxy workspace for analyses of user data and viewing in context with public data already integrated into EuPathDB. Our active user support offers an email help desk, social media, video tutorials and a worldwide program of workshops. These free resources easily merge evidence from diverse data and across species to place the power of bioinformatics with every scientist. Recent expansion includes ClinEpiDB.org, a site which facilitates the exploration and analysis of epidemiologic studies. Our planned future growth includes a merger with VectorBase, home to genome, Omic and population data for many vector species.

The many sides of *Toxoplasma* autophagy machinery

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Toxoplasma gondii is one of the most successful parasites in the world, infecting a wide variety of warm-blooded animals and about one third of the human population. It can cause a disease called toxoplasmosis, but is generally largely asymptomatic in healthy adults. However, infections in immunocompromised individuals have devastating consequences, and can for example lead to lethal encephalitis.

This unicellular parasite possesses an apparently reduced autophagy-related machinery, but is nevertheless able to generate autophagosome-like structures in acute starvation conditions.

Over the last few years, we have discovered that some autophagy-related proteins are also crucial for the function of a parasite-specific plastid called the apicoplast, a metabolically important organelle of endosymbiotic origin. For example, TgATG8 is recruited to the outermost membrane of the plastid, where it plays a role in organelle inheritance during cell division.

Early components of the autophagy pathway such as TgATG9 are likely solely involved in a canonical degradative autophagic pathway: they are not crucial for parasite viability in normal growth conditions, although they are important for sustaining stress conditions, and in the context of parasite differentiation into the latent form of *Toxoplasma*. In contrast, TgATG8 and related membrane-conjugating machinery are important for maintaining the homeostasis of the apicoplast in the course of normal parasite growth, and are thus essential for parasite viability. Interestingly, both canonical and non-canonical functions may thus be exploited for discovering new potential drug targets.

The phosphatases of *Toxoplasma gondii*

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Toxoplasma gondii, an obligate intracellular parasite of the phylum Apicomplexa, infects warm-blooded animals including an estimated two billion people. The propagation and pathogenesis of *Toxoplasma gondii* is the consequence of repeated lytic cycles of parasite attachment to a host cell, invasion, replication within a parasitophorous vacuole, and egress from the cell. This lytic cycle is delicately regulated by calcium-dependent reversible phosphorylation of the molecular machinery that drives invasion and egress. While much progress has been made elucidating the protein kinases and substrates central to parasite propagation, little is known about the relevant protein phosphatases. In the past years we have taken a concerted effort to characterize phosphatases that might play a role in the lytic cycle of *Toxoplasma*. An initial study focused on the five protein phosphatases that are predicted to be membrane-associated either integrally or peripherally determined that PPM5C, a PP2C family member, localizes to the plasma membrane of *Toxoplasma* and regulates parasite attachment. More recently we have characterized the secreted metollophophatase Gra44. Gra44 is secreted into the parasitophorous vacuole within which the parasite divides where it interacts with member of a complex involved in translocation of parasite proteins into the host cell. Current efforts are focused on two parasite specific phosphatases, PPKL and PRL, that localize to cytoskeletal components of dividing parasites. All these studies are allowing us to shed light on the diversity of phosphatases and their functions in the propagation and pathogenesis of Toxoplasma.

Cell death in the human malaria parasite: exploring autophagy as a drug target and early cellular events following drug perturbation

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The emergence and spread of resistance to antimalarial drugs has highlighted the demand to search for new drugs that target novel pathways in the human malaria parasite*Plasmodium falciparum*. Here we explore the potential for targeting of the PfAtg8-PfAtg3 protein-protein interaction (PPI) that is required for lipidation of phosphatidylethanolamine (PE) within the autophagy pathway. Whilst *P. falciparum* lacks a complete cascade of the classical autophagy pathway, the homologues for key proteins such as PfAtg8 exist and have been shown to be involved in cellular processes such as vesicle trafficking and apicoplast biogenesis. Here, the antiplasmodial activity of a library of 131 compounds designed *in silico* to act as inhibitors of hLC3 (Atg8 homologue)-Atg3 interaction were evaluated. Two hits, SK1.47 and SK1.49, show moderate antiplasmodial activity (EC_{50} of 1-2 µM) against intraerythrocytic parasites, produce a rapid cytocidal activity against trophozoite stage parasites and have selectivity for the parasites over HepG2 cells. As a first proof of concept, both compounds inhibit the formation of PfAtg8-labelled vesicles, potential autophagosomes, induced by nutrient starvation. Computational modelling of SK1.47 and SK1.49 docking to PfAtg8 suggests that the naphthalene group binds to the W-pocket and the substituted phenyl binds to the L-pocket of the PfAtg3 interacting region of PfAtg8. This docking study also highlights aspects of the core structure of both molecules that should be further explored in terms of their antiplasmodial activity.

Ultrastructural changes and induction of biochemical markers of apoptotic cell death appear to suggest that SK1.47 and SK1.49 treated parasites do not undergo apoptotic cell death. This study was extended to exploit a bioluminescence assay of parasite viability which allows samples to be prepared that match a defined and titrated kill effect applied using drugs of different chemical classes. These established the conditions for a comparative study of apoptotic markers of early cell death using these different chemical classes, and also highlighted the central role of mitochondrial membrane potential collapse for the majority of drugs that explored and Ca^{2+} redistribution from the digestive vacuole following treatment with 4-aminoquinolines.

This study highlights the opportunity of autophagy related proteins (Atg) of *P. falciparum* parasite as a novel target for drug development. Although SK1.47 and SK1.49 compounds are not considered as lead compounds for drug development due to lack the required potency with unfavourable physicochemical properties (high LogP), they are available now as chemical probes to explore the contested role of autophagy in malaria parasite homeostasis and response to drugs.

Trypanosoma cruzi α-Gal-terminating neoglycoproteins as biomarkers for early assessment of chemotherapeutic outcomes in Chagas disease

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Chagas disease (ChD), caused by the protozoan parasite *Trypanosoma cruzi*, affects millions of people worldwide. Chemotherapy is restricted to two drugs, benznidazole (BZN) and nifurtimox, which are less effective in the chronic stage and may cause severe side effects, leading to cessation of treatment in a significant number of patients. Negative seroconversion using conventional serology assays following chemotherapy takes approximately 10-20 years to occur, which is a very poor prognostic perspective to support the widespread treatment of chronic ChD. Furthermore, there are no clinical, validated biomarkers (BMKs) to assess therapeutic efficacy of these drugs in the chronic stage. Due to these reasons and others, it is estimated that ≤1% of chronic patients undergo treatment. Therefore, lack of reliable BMKs for assessment of therapeutic efficacy following chemotherapy is a major challenge in ChD. The plasma membrane of infective trypomastigote forms of *T. cruzi* is covered by a dense coat of glycosylphosphatidylinositol (GPI)-anchored glycoconjugates, including major glycoprotein families of mucins, mucin-associated-surface proteins (MASP), and *trans*-sialidases (TS). In particular, the highly abundant GPI-anchored mucins of the infective trypomastigote stage (tGPI-mucins) display *O*-glycans containing terminal, nonreducing α-galactosyl (α-Gal) glycotopes, which are absent in human tissues and are, therefore, highly immunogenic to humans. Here, we describe the synthesis of various α-Gal-terminating neoglycoproteins (α-Gal-NGPs) for the early assessment of chemotherapeutic outcomes in ChD. We will discuss in detail the results of different serum panels from cohorts of ChD patients treated with BZN and followed up for different periods. Overall, our data strongly indicate that certain α-Gal-NGPs have a great potential as BMKs for early assessment of therapeutic response in ChD.

Fatal progression of experimental visceral leishmaniasis is associated with intestinal parasitism and secondary infection by commensal bacteria, and is delayed by antibiotic prophylaxis

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Abstract

Leishmania donovani causes visceral leishmaniasis (VL), which is typically fatal without treatment. There is substantial variation between individuals in rates of disease progression, response to treatment and incidence of post-treatment sequelae, specifically post-kala-azar dermal leishmaniasis (PKDL). Nevertheless, the majority of infected people are asymptomatic carriers. Hamsters and mice are commonly used as models of fatal and non-fatal VL, respectively. Host and parasite genetics are likely to be important factors, but in general, the reasons for heterogeneous disease presentation in humans and animal models are poorly understood. Host microbiota has become established as a factor in cutaneous forms of leishmaniasis but this has not been studied in VL. We induced intestinal dysbiosis in mice and hamsters by long-term treatment with broad-spectrum antibiotics in their drinking water. There were no significant differences in disease presentation in dysbiotic mice. In contrast, dysbiotic hamsters infected with *L. donovani* had delayed onset and progression of weight loss. Half of control hamsters had a rapid progression phenotype compared with none of the ABX-treated animals and the nine-month survival rate was significantly improved compared to untreated controls (40% vs. 10%). Antibiotic-treated hamsters also had significantly less severe hepatosplenomegaly, which was accompanied by a distinct cytokine gene expression profile. The protective effect was not explained by differences in parasite loads or haematological profiles. We further found evidence that the gut-liver axis is a key aspect of fatal VL progression in hamsters, including intestinal parasitism, bacterial translocation to the liver, malakoplakia and iron sequestration, none of which occurred in non-progressing murine VL. The results provide experimental support for antibiotic prophylaxis against secondary bacterial infections as an adjunct therapy in human VL patients.

Title: Introducing ECLIPSE: a new intervention programme to improve patient journey and reduce stigma for people with cutaneous leishmaniasis

Helen Price

In this talk I will introduce ECLIPSE, a new four-year applied healthcare programme which aims to improve the patient journey and reduce stigma for people with cutaneous leishmaniasis in endemic communities of Brazil, Ethiopia and Sri Lanka. ECLIPSE brings together expertise in an international, cross-cultural, multidisciplinary team that includes clinicians, anthropologists, psychologists, disease specialist and public health researchers. This new partnership includes established researchers, a cohort of early career researchers who will undertake ground-breaking research and the ECLIPSE Policy Network which brings together policy makers from the three countries to learn from best practice. We will use anthropological and sociological methods to gain in-depth understanding of the effects of CL on the daily lives of those affected and the barriers to seeking healthcare, obtaining accurate, early diagnosis and receiving effective treatment. The insights we gain will inform the development of new interventions: community education campaigns to increase disease awareness and reduce stigma and training packages for healthcare professionals. The interventions will first be tested, refined and then implemented in three field sites in each country. We anticipate that this research will benefit patients and communities in Brazil, Ethiopia and Sri Lanka by increasing early diagnosis and treatment uptake; helping patients improve their quality of life; empowering communities to reduce stigma and social isolation; and ensuring that community health workers are able to help CL patients.